EpiNext™ RRBS Library Fast Kit

Base Catalog # P-1069

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ RRBS Library Fast Kit is designed to prepare reduced representation bisulfite sequencing (RRBS) DNA libraries for an Illumina platform. The optimized protocol and components of the kit allow subnanogram DNA to be used for MSP1 DNA digestion and bisulfite conversion, followed by non-barcoded (singleplexed) or barcoded (multiplexed) library construction in less than 4 hours.

Starting Material and Input amount: Starting materials can be genomic DNA isolated from various tissue/cell samples such as fresh and frozen tissue, cultured cells from a flask or microplate, microdissection samples, FFPE tissue, plasma/serum, and body fluid samples, etc. DNA should be without any previous restriction digestion step. Plasmid DNA can be used for bisulfite treatment with or without previous linearization, as the kit allows for DNA denaturation status to remain during the entire DNA bisulfite conversion process and direct ligation of adaptors to bisulfite DNA. Input amount of DNA can be from 10 ng to 400 ng. For optimal preparation, the input amount should be 200 ng.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tube/vials. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	12 reactions Cat. #P-1069-12	24 reactions Cat. #P-1069-24	Storage Upon Receipt
MSPI (20 U/µl)*	26 µl	52 µl	-20°C
10 X Digestion Buffer*	40 µl	80 µl	-20°C
Modification Buffer	3 ml	6 ml	RT
Modification Powder	2 vials	4 vials	RT
DNA Purification Buffer	1.5 ml	3 ml	RT
Desulphonation Solution*	50 µl	100 µl	RT
Elution Solution	0.5 ml	1 ml	RT
F-Spin Column	15	30	RT
F-Collection Tube	15	30	RT
5X Reaction Buffer*	100 µl	200 µl	-20°C
Reaction Enzyme Mix*	50 µl	100 µl	-20°C
3'-Adaptor (10 µM) *	28 µl	56 µl	-20°C
5'-Adaptor (10 µM) *	28 µl	56 µl	-20°C
MQ Binding Beads	1.8 ml	2 X 1.8 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	RT

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C.

Upon receipt: (1) Store the following components at -20°C immediately: **MSPI, 10 X Digestion Buffer**, **5X Reaction Buffer, Reaction Enzyme Mix**, **3'-Adaptor**, **5'-Adaptor**, **2X HiFi PCR Master Mix**, **Primer U** and **Primer I**. (2) Store the following components at 4°C: **MQ Binding Beads**. (3) Store all other components at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Agilent® Bioanalyzer® or comparable method to assess the quality of the DNA library
- ☐ Thermocycler
- ☐ Centrifuge including desktop centrifuge (up to 14,000 rpm)

- ☐ 96-well format magnetic stand (e.g., EpiMag HT (96-Well) Magnetic Separator, EpigenTek's Cat. No. Q10002)
- ☐ Pipettes and pipette tips
- ☐ PCR tubes or plates
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 90% Ethanol
- ☐ Isopropanol (100%)
- ☐ Distilled or deionized water
- ☐ DNA sample

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiNext™ RRBS Library Fast Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiNext™ RRBS Library Fast Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ RRBS Library Fast Kit and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

DNA methylation occurs by the covalent addition of a methyl group (CH3) 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 the 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 targets used for therapeutics.

Reduced representation bisulfite sequencing (RRBS) is the most used genome-wide DNA methylation analysis method. It enriches CpG-rich DNA fragments with restriction enzyme digestion and converts unmethylated cytosine to uracil, while 5-mC remains unmodified by the bisulfite treatment. Epigenetic differences are interpreted as genetic differences, which can then be detected by sequencing at single-base resolution and on a genome-wide scale. However, traditional RRBS is still not practical for many reasons. (1) It requires relatively large amounts of DNA as input material, which is difficult to prepare from limited biological samples such as tumor biopsy, early embryos, embryonic tissues, and circulating DNA. (2) DNA must be sheared first 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, resulting in the formation of mono-tagged templates that will be removed during library enrichment. Thus, incomplete coverage and bias occur when performing RRBS. (3) The method is also time-consuming, taking approximately 2 days. To overcome the weaknesses of these methods, EpigenTek offers the EpiNext™ RRBS Library Fast Kit. This kit has the following features:

- **Innovative method:** Allows for rapid size-appropriate CpG-rich DNA fragment preparation and bisulfite conversion. The bisulfite DNA can be directly ligated to adaptors thereby eliminating the possibility of breaking adaptor-ligated fragments, which often occurs with currently used RRBS methods.
- **Fast and streamlined procedure:** The procedure from DNA enzyme digestion to ready-to-use library DNA can be completed within 4 hours.
- **Complete conversion:** Completely converts unmethylated cytosine into uracil (>99%) with negligible inappropriate- or error-conversions of methylcytosine to thymine.
- **High sensitivity and efficiency:** Innovative adaptor ligation of bisulfite DNA eliminates loss of fragments and selection bias, enabling input DNA to be as low as 10 ng, making it ideal for methylation profiling of precious, limited samples. The kit can be used for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation.
- **Extremely convenient:** The kit contains all required components for each step of DNA library preparation, which are sufficient for MSPI digestion, 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 tissue, cultured cells from a flask or microplate, microdissection samples, paraffin-embedded tissue, biopsy, embryonic cells, plasma/serum samples, and body fluid samples.

PRINCIPLE & PROCEDURE

This kit includes all reagents required for successfully preparing a library directly from bisulfite DNA generated from a tiny amount of input DNA. In this preparation, size-appropriate CpG-rich DNA fragments are enriched by MSPI digestion and bisulfite converted. The bisulfite-treated DNA, in single-stranded form, is then simultaneously converted to dsDNA and adaptor-ligated. Next, the ligated fragments are size-selected and purified using **MQ Binding Beads**, followed by amplification with a high-fidelity PCR Mix. The procedure ensures maximum yields from minimum amounts of starting material and provides highly accurate amplification of library DNA with low error rates and minimum bias.

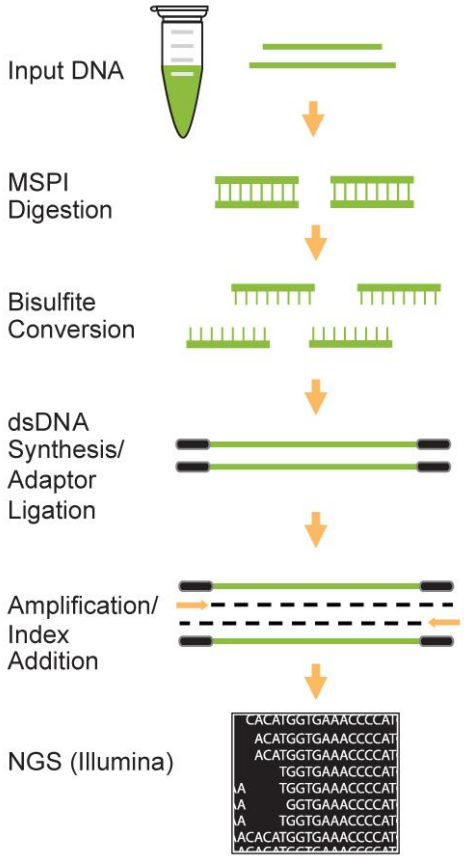

Fig 1. Workflow of the EpiNext™ RRBS Library Fast Kit

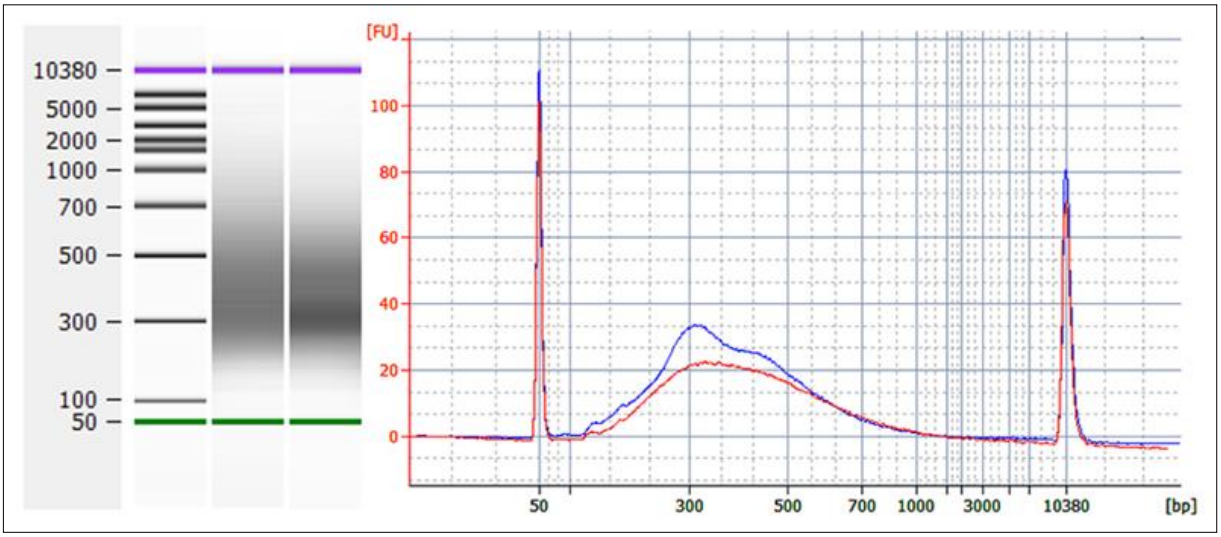

Fig 2. Size distribution of library fragments. RRBS DNA libraries were prepared from human Hela DNA using the EpiNext™ RRBS Library Fast Kit: Input DNA: 20 ng (red trace), 200 ng (blue trace). Fragment size is from 160-800 bps with peak size at 320 bps.

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input DNA Amount: DNA amount can range from 10 ng to 400 ng per reaction. An optimal amount is 200 ng per reaction. Starting DNA may be in water or in a buffer such as TE. DNA should be high quality and relatively free of RNA. RNase I can be used to remove RNA.

DNA Isolation: Use your method of choice for DNA isolation. EpigenTek offers a series of genomic DNA isolation kits for your convenience.

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

Procedure overview and time table

Steps	Required time
MSP1 digestion and purification*	20 min
Bisulfite DNA conversion and purification*	80 min
dsDNA conversion and purification	35 min
DNA ligation and purification*	40 min
Library amplification and purification*	50 min

**Stop point. DNA generated at the end of this step can be stored at -20°C for future use.*

1. MSPI Digestion

a. Prepare the MSPI digestion reaction in a 0.2 ml PCR tube according to table 1:

Table 1. MSPI digestion

Component	Volume
DNA	2-10 µl (200 ng)
10X Digestion Buffer	2 µl
MSPI (20 U/µl)	2 µl
H2O	6-14 µl
Total volume	**20 µl**

b. Mix and incubate at 37° C for 15 min.

Note: *Incubation time can be also safely extended as long to overnight at 37° C if needed.*

c. Resuspend **MQ Binding Beads** by vortex and add exactly 8 µl (0.4X) of resuspended beads to the PCR tube of MSPI digestion reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

***Note**: 1). 0.4 X MQ beads generally allow to collect 40-800 bp DNA in supernatant. 2). To ensure the correct ratio of **MQ Binding Beads** to sample solution during DNA clean up, make sure that any bead solution stuck on the outside of the pipette tip is removed before adding beads into the sample vial.*

d. Incubate for 5 minutes at room temperature to allow large DNA fragments to bind to beads.

e. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully transfer the supernatant to a new 0.2 ml PCR tube for bisulfite conversion. Be careful not to disturb the beads.

2. Bisulfite DNA Modification

a. 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 (a trace amount of undissolved **Modification Powder** may remain, which is normal as **Modification Powder** is saturated in solution).

b. For each 0.2 ml PCR tube containing MSPI digestion sample supernatant, add 140 µl of the mixed **Modification Solution**.

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

95°C 2 min
60°C 30 min] *X 2 cycles*

4°C hold for up to 16 h

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

3. Converted DNA Clean-Up

a. Prepare **DNA Binding Solution** by adding 100 ul of **DNA Purification Buffer** to 200 ul of Isopropanol per sample.

b. Add 300 µl of **DNA Binding Solution** to each column. Then transfer the sample from each PCR tube (from Step 2) to each column containing the **DNA Binding Solution**. Sit at room temperature for 1 min to mix the sample and **DNA Binding Solution.**

c. Centrifuge at 12,000 rpm for 1 min. Remove columns from collection tubes and discard the flowthrough. Place columns back into collection tubes.

d. Prepare final desulphonation buffer by adding 30 µl of **Desulphonation Solution** to every 1 ml of *90% ethanol*, and mix. Add 100 µl of the final desulphonation buffer (**Desulphonation Solution** and *90% ethanol* mixture) to each column. Allow columns to sit for 10-15 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.

e. 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 1 min.

f. Insert each column into a new 1.5 ml tube. Add 12 µl of **Elution Solution** directly to each column's filter membrane. Centrifuge at 12,000 rpm for 60 sec to elute converted DNA.

Converted 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 150-200 bps.

***Note**: To ensure the DNA is properly converted, we recommend checking the bisulfite-treated DNA by real time methylation-specific PCR (MS-PCR). For your convenience and the best results, EpigenTek provides Methylamp™ MS-qPCR Fast Kit (Cat. No. P-1028) for real time MS-PCR. Both positive primers (b-actin, component of kit Cat. No. P-1028) and negative primers (GAPDH, component of kit Cat. No. P-1029) are also separately available for checking conversion efficiency.*

4. dsDNA Conversion

a. Prepare dsDNA Conversion reaction in 0.2 ml PCR tube according to Table 2:

Table 2. dsDNA Conversion

Component	Volume
Converted DNA (from Step 3) *	10 µl
5X Reaction Buffer	3.5 µl
3'-Adaptor (10 µM)	2 µl
Reaction Enzyme Mix	2 µl
Total volume	**17.5 µl**

***** *If converted DNA volume is less than 10 µl, add distilled water to make the total volume 17.5 µl.*

b. Mix and incubate for 20 min at 37°C in a thermocycler without heated lid (make sure to set lid temperature to 25°C).

5. Clean-Up of dsDNA

***Note**: To ensure the correct ratio of **MQ Binding Beads** to sample solution during DNA clean up, make sure that any bead solution stuck on the outside of the pipette tip is removed before adding beads into the sample vial.*

a. Resuspend **MQ Binding Beads** by vortex.

b. Add exactly 21 µl of resuspended beads to the PCR tube of dsDNA synthesis reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

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

d. 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 not to discard the beads that contain DNA.

e. Keep the PCR tube in the magnetic stand and add 150 µl of freshly prepared *90% ethanol* to the tube, and then carefully remove and discard the ethanol.

f. Repeat Step 5e once for a total of two washes.

g. Air dry beads for 1 minute while the tube is on the magnetic stand.

h. Resuspend the beads in 10.5 µl **Elution Buffer**, and incubate at room temperature for 4 minutes to release the DNA from the beads.

i. Capture the beads by placing the tube in the magnetic stand for 1 minute or until the solution is completely clear

j. Transfer 10 µl of clear solution to a new 0.2 ml PCR tube for ligation.

6. DNA ligation

a. Prepare ligation reaction in a 0.2 ml PCR tube according to Table 3:

Table 3. DNA Ligation

Component	Volume
dsDNA (from Step 5)	10 µl
5X Reaction Buffer	3.5 µl
5'-Adaptor (10 µM)	2 µl
Total volume	**15.5 µl**

b. Mix and incubate for 2 min at 98°C in a thermocycler without heated lid (make sure to set lid temperature to 25°C) followed by incubation on ice for 2 min. Add 2 µl of **Reaction Enzyme Mix** and then incubate at 37°C for 20 min in a thermocycler without heated lid.

7. Clean-Up of Ligated DNA

***Note**: To ensure the correct ratio of **MQ Binding Beads** to sample solution during DNA clean up, make sure that any bead solution stuck on the outside of the pipette tip is removed before adding beads into the sample vial.*

a. Resuspend **MQ Binding Beads** by vortex.

b. Add exactly 21 µl of resuspended beads to the tube of the ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

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

d. 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 not to discard the beads that contain DNA.

e. Keep the PCR tube in the magnetic stand and add 150 µl of freshly prepared *90% ethanol*. Then carefully remove and discard the ethanol.

f. Repeat Step 7e one more time for a total of two washes.

g. Air dry beads for 1 minute while the tube is on the magnetic stand.

h. Resuspend the beads in 11 µl **Elution Buffer**, and incubate at room temperature for 4 minutes to release the DNA from the beads.

i. Capture the beads by placing the tube in the magnetic stand for 1 minute or until the solution is completely clear.

j. Transfer 10.5 µl of clear solution to a new 0.2 ml PCR tube for library amplification and indexing.

8. Library Amplification & Indexing

a. Prepare the PCR Reactions:

Thaw all reaction components including master mix and primer solution. 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 Table 4:

Table 4. Library Amplification and Indexing

Component	Volume
HiFi Master Mix (2X)	12.5 µl
Primer U	1 µl
Primer I (or barcode index)	1 µl
Ligated DNA (from Step 7)	10.5 µl
Total Volume	**25 µl**

Important Note: (1) *Use of **Primer I** included in the kit will generate a singleplexed library. For multiplexed library preparation, replace **Primer I** with one of the12 different barcodes (indexes) contained in the EpiNext™ NGS Barcode (Index) Set-12 (Cat. No. P-1060) to generate each indexed library. You can also add user-defined barcodes (Illumina compatible) instead of **Primer I**. (2) Each indexed library can be combined in equal amounts to form multiplexed libraries for sequencing. (3) The amount of indexed library can be quantified using qPCR, Qubit or Picogreen assays.*

b. Program the PCR Reactions:

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

Cycle Step	Temp	Time	Cycle
Activation	98°C	30 sec	1
Cycling	98°C 55°C 72°C	10 sec 20 sec 20 sec	vary*
Final Extension	72°C	2 min	1

** PCR cycles may vary depending on the input DNA amount. In general, use 20 cycles for 200 ng, 21 cycles for 100 ng, and 24 cycles for 10 ng of input DNA. Further optimization of PCR cycle number may be required.*

9. Clean-Up of Amplified Library

***Note**: To ensure the correct ratio of **MQ Binding Beads** to sample solution during DNA clean up, make sure that any bead solution stuck on the outside of the pipette tip is removed before adding beads into the sample vial.*

a. Resuspend **MQ Binding Beads** by vortex.

b. Add exactly 20 µl (0.8X) of resuspended beads to the amplified library. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

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

d. 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 not to discard the beads that contain DNA.

e. Keep the PCR tube in the magnetic stand and add 150 µl of freshly prepared *90% ethanol* to the tube. then carefully remove and discard the ethanol.

f. Repeat Step 9e one more time for a total of two washes.

g. Air dry beads for 1 minute while the tube is on the magnetic stand.

h. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 4 minutes to release the DNA from the beads.

i. Capture the beads by placing the tube in the magnetic stand for 1 minute or until the solution is completely clear.

j. Transfer 12 µl to a new 0.2 ml PCR tube for immediate use or store at -20°C until ready to use for sequencing.

***Note:** (1) Quality of the prepared library can be assessed using TapeStation, Agilent® Bioanalyzer® or comparable method. Library fragments should have the correct size distribution (ex: 200 bps at peak size) without adaptors or adaptor-dimers (about 127 bps). (2) To check the size distribution,*

dilute library with water (if necessary) and apply it to an Agilent high sensitivity chip. If there is the presence of <150 bp adaptor dimers, it is recommended to use 0.8X ***MQ Binding Beads*** *to remove fragments below 150 bps. (3) The amount of indexed library can be quantified using qPCR, Qubit or Picogreen assays. (4) Each indexed library can be combined in equal amounts to form multiplexed libraries for sequencing.*

SEQUENCING & BIOINFORMATICS CONSIDERATIONS

Libraries prepared with this kit are compatible with Illumina sequencing platforms. These RRBS libraries are built using post-bisulfite adaptor tagging, adapted from whole genome bisulfite sequencing (WGBS) methods, to improve read generation from very low amounts of DNA. Take note of following sequencing recommendations:

- Because RRBS libraries have short fragments, single-read sequencing with 50 bp read length (SR50) is recommended for the best cost to budget ratio, although longer reads and paired end reads are possible with proper trimming.
- Enzymatic digestion of RRBS libraries have bias with the same starting bases, which could reduce clustering efficiency of sequencing and thus a PhiX spike-in control of 5%-20% is recommended, depending on the sequencer's model (refer to its instruction manual) to improve library diversity.
- The number of libraries pooled for multiplexed sequencing is dependent on the desired sequencing depth, sequencing instrument, and genome size of species. For reference, the typical reads necessary for human or mouse species at a minimum of 5X-10X sequencing depth are 20-30 million SR50 reads.

The recommended bioinformatics pipeline is as follows:

- Quality and adapter trimming on raw FASTQ reads using Trim Galore by removing low-quality reads with a Sanger Phred score of 20 or lower and trimming the 3' Illumina TruSeq type adapter (AGATCGGAAGAGC). Use the --rrbs flag in Trim Galore to discard the first 2 biased bases due to restriction enzyme digestion, and optionally add --paired flag if it is a paired end read. Use --non_directional flag.
- De-duplication is not recommended as it may discard reads of areas where high coverage of a region is expected.
- Align trimmed reads to the reference genome using methylation-aware mapper Bismark package with the --non_directional flag (--pbat may also be used, although this is intended for WGBS and mapping efficiency differences will be negligible).
- MethylKit can be used to extract the methylation information extracted from the Bismark mapping results in the CpG context. Differentially methylated analysis typically uses a minimum 5 coverage setting, with any detected bases of 10 or higher considered to be good.

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
DNA is poorly converted	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 gel. Ensure that RNA is removed by RNase treatment.
	Too little DNA or too much DNA (i.e., <5 ng or >500 ng).	Increase or decrease input DNA to within the correct range, or to the optimal amount of 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 **Desulphonation Solution** is added into every 1 ml of *90% ethanol* in Step 3d.
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 3b.
	Incorrect concentration of ethanol solution used for DNA clean-up	Use *90% ethanol* for DNA clean-up.
	Sample did not completely pass through the column filter membrane.	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 MSPI digestion should be 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 dsDNA conversion, library synthesis, and library amplification & indexing.
	Improper storage of the kit.	Ensure the kit has not exceeded the expiration date. Standard shelf-life is 6 months from date of receipt when stored properly.
Presence of <150 bp adaptor dimers	Improper ratio **of MQ Binding Beads** to DNA volume in size selection.	Check if the correct volume of **MQ Binding Beads** is added to the DNA solution accordingly. Use 0.8X **MQ Binding Beads** to remove fragments below 150 bps.
	Insufficient ligation.	Too little input DNA and too much adaptors may cause insufficient ligation and adaptor dimers. Make sure that ligation reaction is processed with the proper amount of input DNA and adaptors.
	Over-amplification of library.	PCR artifacts from over-amplification of library may cause increased adaptor dimers. Make sure to use proper PCR cycles to avoid this problem.

RELATED PRODUCTS

DNA Isolation and Cleanup

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1004	FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006	DNA Concentrator Kit
P-1009	FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017	FitAmp™ Urine DNA Isolation Kit
Q10002	EpiMag HT (96-Well) Magnetic Separator
P-1018	FitAmp™ Blood and Cultured Cell DNA Extraction Kit

DNA Bisulfite Conversion

P-1001	Methylamp™ DNA Modification Kit

P-1026	BisulFlash™ DNA Modification Kit

PCR Analysis

P-1028	Methylamp MS-qPCR Fast Kit

DNA Library Preparation

P-1051	EpiNext™ DNA Library Preparation Kit (Illumina)
P-1053	EpiNext™ High-Sensitivity DNA Library Preparation Kit (Illumina)
P-1056A	EpiNext™ High-Sensitivity Bisulfite-Seq Kit
P-1059	EpiNext™ DNA Size Selection Kit
P-1063	EpiNext™ DNA Purification HT System

NGS Barcode

P-1060	EpiNext™ NGS Barcode (Index) Set-12

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