



July 2022

# QIAseq<sup>®</sup> Methyl Library Handbook

For DNA library construction for whole genome bisulfite sequencing on Illumina<sup>®</sup> sequencing platforms

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# Kit Contents

## Box 1

<b>QIAseq Methyl Library Kit</b>	<b>(24)</b>
<b>Catalog no.</b>	<b>180502</b>
<b>No. of preps</b>	<b>24</b>
VeraSeq Ultra DNA Polymerase	15 µL
VeraSeq Buffer II, 5X	500 µL
RNase-free Water	5 x 1.9 mL
Primer Mix Illumina Libr. Amp	2 x 20 µL
dNTP Mix (10 mM/each)	55 µL
BisJ DNA repair Enzyme Mix	60 µL
BisJ DNA repair Buffer, 5X	500 µL
Ultralow Input Ligase	3 x 65 µL
Ultralow Input Ligation Buffer, 4X	900 µL
Quick-Start Protocol	1

<b>QIAseq UDI Y-Adapter Kit (24)</b>	
<b>Catalog no.</b>	<b>1118256</b>
QIAseq UDI Y-Adapter Plate (24)*	1
UDI Y-Adapter Plate (24) Reference Card	1

\* Barcode: UDI 1–24.

## Box 2

<b>QIAseq Methyl Library Kit</b>	<b>(24)</b>
<b>Catalog no.</b>	<b>180502</b>
<b>No. of preps</b>	<b>24</b>
QIAseq Beads	5 mL

**Note:** QIAseq library kits can be combined with any compatible QIAseq Y-adapter kit. Y-adapter kits are available as unique dual-index (UDI) or combinatorial dual-index (CDI) solutions for 24 and 96 reactions and for multiplexing of up to 384 samples. See Ordering Information.

## Shipping and Storage

The QIAseq<sup>®</sup> Methyl Library Kit is shipped in two boxes. Box 1 is shipped on dry ice or blue ice and Box 2 is shipped at room temperature (15–25°C). Upon receipt, all components in Box 1 should be stored immediately at –30°C to –15°C in a constant-temperature freezer. All components in Box 2 should be stored immediately at 2–8°C.

## Intended Use

The QIAseq Methyl Library Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN<sup>®</sup> products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

## Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at [www.qiagen.com/safety](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN kit and kit component.

## Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAseq Methyl Library Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

Epigenetics describes the study of heritable changes in gene function that occur without a change in the nuclear DNA sequence. In addition to RNA-associated silencing and histone modification, a major epigenetic mechanism in higher-order eukaryotes is DNA methylation. Epigenetic changes play a crucial role in the regulation of important cellular processes, such as gene expression and cellular differentiation, and were also identified as key factors in many various diseases.

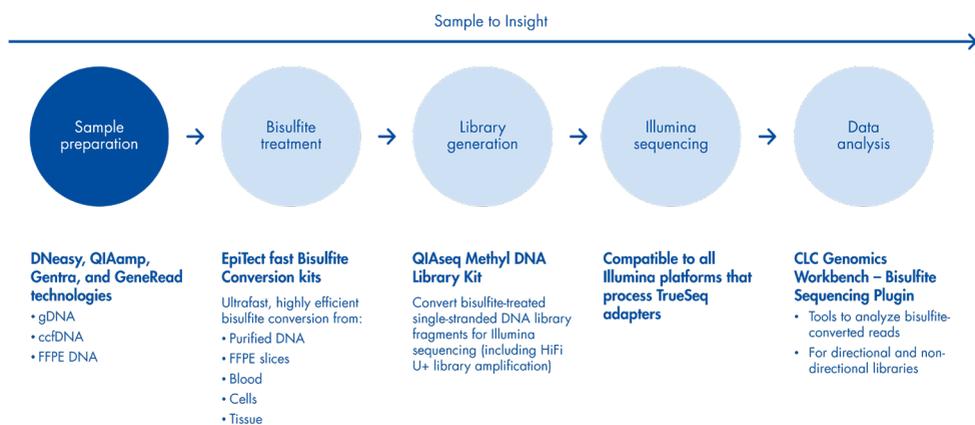
DNA methylation occurs on cytosine residues, especially in CpG islands, which are GC-rich regions. They are usually clustered around the regulatory region of genes and can affect their transcriptional regulation. Methylation of CpG islands is known to inactivate gene expression and plays an important role in normal and disease development. Cytosine methylation may also occur in non-CpG content, as described for embryonic stem cells.

Whole genome bisulfite sequencing (WGBS), which combines bisulfite-mediated conversion of unmethylated cytosines to uracil and next generation sequencing (NGS), allows genome-wide detection of 5-methylcytosine residues at unprecedented single-base resolution. In turn, this enables the connection of gene activity and the precise localization of a DNA methylation marker.

Clinically relevant specimens for next-generation sequencing can be difficult to obtain or yield limited amounts of nucleic acids. Therefore, researchers wish to use samples as efficiently as possible. Solutions that push the limits of input amounts – without sacrificing sensitivity and performance – are needed to safeguard samples. However, current approaches used to treat samples with bisulfite after NGS library preparation in order to convert unmethylated cytosines to uracils reveal major challenges, such as significant bisulfite-induced sample loss due to DNA degradation. Traditional library preparation methods for bisulfite sequencing demand very high DNA input amounts, since they require several purification steps or require a large number of PCR cycles during NGS library construction.

The QIAseq Methyl Library procedure delivers a fast and streamlined workflow for NGS library construction starting from bisulfite-converted single-stranded DNA. The protocol consists of a two-step reaction performed in a single tube, with no intermediate purification steps. This approach reduces both DNA loss and the need for higher DNA input. This post-bisulfite library construction protocol does not require extra methylated adapters. Furthermore, optimized enzymes and buffer compositions ensure superior yields of high-quality, NGS-ready libraries – even from low input.

The QIAseq Methyl Library chemistry applies highly efficient priming and double-strand synthesis of the bisulfite-converted single strands, combined with end-polishing of the generated double strand in only one step followed by ultra-efficient adapter ligation. Library amplification uses the VeraSeq Ultra DNA Polymerase which is a high-fidelity enzyme that tolerates the presence of uracil. This is important, since evaluation of errors after bisulfite sequencing is difficult, due to variability that originates from methylation from incomplete conversion and errors introduced during sequencing. Dedicated EpiTect Fast bisulfite conversion protocols deliver complete conversion of unmethylated cytosines and optimal starting material for library generation, without the need for extra fragmentation. After library construction and sequencing on Illumina platforms, bioinformatic analysis is extremely simplified using the Bisulfite Sequencing Plug-in (version 2.0 or later) for the Biomedical Genomics Workbench and CLC Genomics Workbench for non-directional libraries. The entire WGBS workflow is shown in Figure 1.



**Figure 1. Whole genome bisulfite sequencing (WGBS) workflow.**

With the QIAseq Methyl Library Kit, reaction setup is straightforward and handling time is greatly reduced, allowing DNA amplification and library preparation to be completed in less than 5 hours. The kit provides a time-saving, one-tube library preparation protocol that does not require extra DNA fragmentation and sample cleanup between steps, minimizing starting material loss and cross-contamination risk. Co-optimization of bisulfite conversion and library construction processes enables a highly streamlined and efficient protocol and generates a ready-to-use WGBS library in under a day.

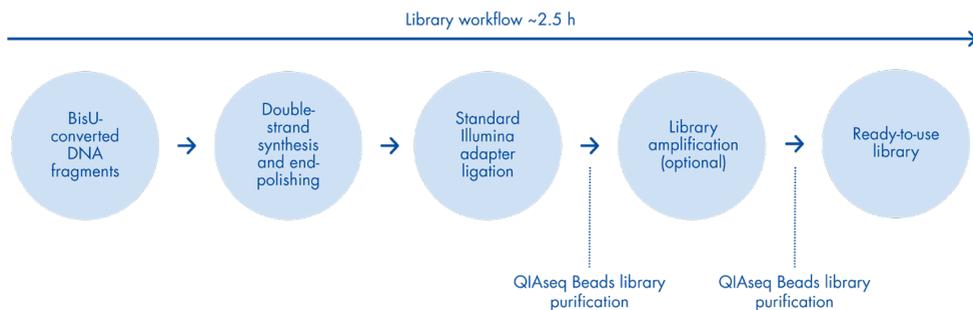
The QIAseq Methyl Library Kit introduces priming of optimized, short primers to enormously reduce starting bias and capture all genomic regions with high efficiency. The high conversion efficiency results in high yields of library without the need for high numbers of library amplification cycles, even from low input. The kit allows the construction of highly complex libraries from a wide range of DNA amount and materials with a high percentage of mapped reads, uniform genome coverage for accurate methylation analysis.

Dual-barcoded, plate-format adapters are included with the QIAseq Methyl Library Kit and allow sequencing on Illumina platforms.

Typical library concentrations from a QIAseq Methyl Library Kit reaction are 2–40 nM with an average fragment size of approximately 350 bp, depending on DNA input and cycle number during library amplification.

## Principle and procedure

The QIAseq Methyl Library Kit uses an enzyme mix and random, short primers for double-strand synthesis of the single-stranded, bisulfite-treated DNA. In the same reaction setup, enzymes repair and prepare the ends of the fragments for subsequent adapter ligation. Both DNA repair and ligation reactions occur in the same tube, minimizing sample loss. The ligation step is followed by a purification step using QIAseq Beads which are included in the kit. Library amplification is performed using the high-fidelity enzyme VeraSeq Ultra DNA Polymerase (Figure 2). VeraSeq Ultra DNA Polymerase is an engineered, ultra-thermostable, uracil-tolerate polymerase designed to maximize the speed, accuracy, and length of DNA synthesis during sequencing template preparation. The result is a novel enzyme that can read through uracil, extend a kilobase of sequence in 15 seconds, and is 25 times more accurate than *Taq* DNA Polymerase. This enables the amplification of WGBS libraries without sequence bias.



**Figure 2. QIAseq Methyl Library workflow.**

Following library construction, the reaction cleanup and removal of adapter–dimers can be achieved using QIAseq Beads. After library amplification, a final cleanup step is performed with QIAseq Beads.

## Description of protocols

This handbook contains protocols for the generation of libraries using bisulfite-converted gDNA, amplification of the libraries, and dedicated protocols for bisulfite conversion of gDNA and prefragmented samples (approximately 200 bp) or ccfDNA using EpiTect Fast Bisulfite Conversion kits (cat. nos. 59802, 59824, 59864). The protocol for library construction is described in “Protocol: Library Generation from Bisulfite-Converted DNA”, page 15. The subsequent library amplification is described in section “Protocol: Library Amplification”, page 20. The dedicated protocols for WGBS using EpiTect Fast DNA Bisulfite Kit are described in “Appendix C: Bisulfite Conversion of Purified gDNA and FFPE DNA Using EpiTect Fast DNA Bisulfite Conversion Kits”, page 32, and “Appendix D: Bisulfite Conversion of Prefragmented (~200 bp) and ccfDNA Using EpiTect Fast Bisulfite Conversion Kits”, page 36. For additional protocols for direct lysis of cells, blood, and FFPE slices, please consult the *EpiTect Fast Bisulfite Conversion Handbook* and use the cycling conditions recommended in “Appendix C: Bisulfite Conversion of Purified gDNA and FFPE DNA Using EpiTect Fast DNA Bisulfite Conversion Kits”.

The generated library can be quantified using qRT-PCR and is optimized for use on Illumina sequencing platforms.

Compatible sequencing platforms:

- Illumina NovaSeq™
- Illumina HiSeq®
- Illumina MiSeq®
- Illumina NextSeq®

## NGS adapter and index technologies

Sample multiplexing is one of the most important NGS tools for increasing throughput and reducing costs. It works by combining multiple samples to be processed together in a single sequencing run; consequently, sequencing reads need to be demultiplexed by reassigning

each single read to its original source library. This is facilitated by the integration of index sequences into the individual adapter molecules.

Recent years have seen increased awareness on the molecular recombination of sample indices (also known as index hopping, index bleeding, etc.). Index hopping mostly occurs during cluster generation and is more common on patterned flow cells that use Illumina's exclusions amplification technology. As a result, indices are exchanged, causing single library fragments to carry a wrong barcode, for example from another library pool. Unique Dual-Indexing technology allows filtering reads with aberrant index pairs from output data to effectively eliminate misassigned reads from downstream analyses.

## QIAseq Dual-Index Y-Adapter Kits

We recommend using QIAseq Dual-Indexed Y-Adapter Kits in combination with the QIAseq Methyl Library Kit. Dual-barcoded plate-format Y-Adapters are available as unique (UDI) or combinatorial dual-indexing (CDI) solution for Illumina instruments. Both types of single-use adapter plates are available for 24 and 96 reactions and have to be purchased separately.

Combinatorial Dual-Index Adapters use combinations of twelve i7 and eight i5 differentially barcoded adapters to enable sample multiplexing of up to 96 samples per sequencing run. In contrast, QIAseq Unique Dual-Index Adapters use 192 unique ten-nucleotide motifs to define 96 sample indices. Each single index motif is only used once on any UDI Adapter plate and is never shared with any motif within another index combination. Accordingly, exchange of index motifs due to index hopping events will result in invalid index pairs. This effectively mitigates the risk of read misassignment during demultiplexing and enables highest accuracy of output data.

Unique Dual-Index Adapter plates are available in 24- and 96-plex format. Combining QIAseq Unique Dual-Index Y-Adapter Kits A–D enables multiplexing of up to 384 samples per sequencing run. For more information on QIAseq Dual-Index Y-Adapters and index sequence motifs see Appendix B: QIAseq Dual-Index Y-Adapters and Ordering Information.

# Equipment and Reagents to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- EpiTect Fast Bisulfite Conversion Kit (QIAGEN, cat. nos. 59802, 59824, 59864)
- PCR tubes or plates
- LoBind® tubes for storage of generated libraries (Eppendorf, cat. no. 0030108094, 0030108116 or 0030108132) or equivalent
- Pipettes and pipette tips
- Magnetic racks for magnetic bead separation (e.g., DynaMag™-2 Magnet, Thermo Fisher Scientific, cat. no. 12321D\*)
- Thermocycler
- Microcentrifuge
- Vortexer
- NanoDrop® UV-Vis spectrophotometer for DNA and bisulfite-treated DNA quantification
- Ice
- 96–100% ethanol
- Agilent® 2100 Bioanalyzer\* or similar to evaluate the DNA fragmentation profile, or comparable capillary electrophoresis device, or method to assess the quality of the DNA library
- QIAseq Library Quant Assay Kit (QIAGEN, cat. no. 333314) for library quantification

\* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

# Important Notes

## DNA preparation and quality control

High-quality DNA is essential for obtaining good bisulfite conversion, library generation, and sequencing results. The most important prerequisite for any DNA sequence analysis experiment is consistent, high-quality DNA from every experimental sample. Therefore, DNA purification procedures, as well as accurate quantification and quality control, are critical to the success of the experiment.

## General precautions

Use good laboratory practices to minimize cross-contamination of nucleic acid products.

Always use PCR tubes, microcentrifuge tubes, and pipette tips that are certified sterile, DNase- and RNase-free.

Before starting, wipe down work area and pipettes with an RNase and DNA cleaning product such as RNase Away<sup>®</sup> (Thermo Fisher Scientific) or LookOut<sup>®</sup> DNA Erase (Sigma-Aldrich).

For consistent bisulfite conversion, library construction and amplification, ensure that the thermal cycler used in the protocol is in good working order and has been calibrated according to the manufacturer's specifications.

Read the entire protocol before starting. Take note of stopping points where samples can be frozen at  $-20^{\circ}\text{C}$  and plan your workflow accordingly.

Library generation is based on a number of enzymatic reactions and is sensitive to many factors such as reaction temperature, time and setup conditions, alcohol contamination, as well as the purity and quality of the input DNA.

When working with plates, we recommend mixing by pipetting samples up and down and changing tips to avoid cross-contamination.

## Recommended library quantification method

QIAGEN's QIAseq Library Quant Assay Kit (cat. no. 333314), which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared library. Alternatively, Qubit® or Agilent Bioanalyzer may be used, but they are not recommended for non-enriched libraries, and are less accurate since they measure all fragments (adapter-ligated, non-adapter-ligated, and ligated fragment with only one adapter).

# Protocol: Library Generation from Bisulfite-Converted DNA

This protocol is for NGS library preparation from bisulfite-converted, single-stranded, and fragmented DNA.

## Important points before starting

- This protocol is optimized for bisulfite-converted DNA using EpiTect Fast Bisulfite Conversion kits, but can be used with bisulfite conversion methods that generate single-stranded DNA with fragment mean size between 200–1500 bp.
- Samples of 10 pg – 500 ng gDNA (optimal: 1–100 ng) as input in bisulfite conversion can be used for library generation using the QIAseq Methyl Library Kit.
- If starting with a low input level, note that quantification of single-stranded DNA after bisulfite conversion will not be accurate.
- Avoid DNA contamination of reagents by using separate laboratory equipment (e.g., pipettes, filter pipette tips, reaction vials).
- Enzymes and enzyme mixes should be thawed on ice. All other components can be thawed at room temperature (15–25°C) but placed on ice immediately after thawing.
- If using less than 500 pg input, the libraries will show lower mapping efficiencies.
- Set up all reactions on ice.

## Things to do before starting

- Thaw BisU DNA Repair Buffer, 5x and Ultralow Input Ligation Buffer, 4X and place on ice.
- Prepare fresh 80% ethanol.
- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- All enzyme mixes should be placed on ice until use.
- Program the thermal cycler. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved on a thermal cycler in advance.
- Equilibrate QIAseq Beads at room temperature. Mix thoroughly before use.

## Procedure

1. Before starting with the setup of the reactions and reagent mixes, program a thermal cycler with the protocols described in Table 1 and Table 2.

**Table 1. Thermal cycling for BisU DNA repair reactions**

Step	Temperature	Incubation Time
1	30°C	30 min
2	68°C	15 min
3	4°C	Hold

**Note:** Use thermocycler with a heated lid set at 75°C.

**Table 2. Thermal cycling for ligation of adapters to end-polished fragments**

Step	Temperature	Incubation Time
1	20°C	15 min
2	4°C	Hold

**Note:** Use thermocycler with lid off.

## BisU DNA repair reaction

2. Prepare bisulfite-converted DNA dilution 10 pg – 500 ng (optimal: 1–100 ng) in RNase-free Water. If no quantification occurs between the EpiTect Fast protocol and the library preparation, use the total volume of the EpiTect Fast Eluate (approximately 16  $\mu$ L).

**Note:** During bisulfite conversion, desulfonation, and column purification of bisulfite-converted DNA, approximately 30–50% of DNA will be lost. Please calculate DNA input accordingly to begin with optimal input for library preparation.

3. Set up the BisU DNA repair reaction mix on ice according to Table 3 and mix by gently vortexing. Keep on ice until further processing.

**Note:** If working with more samples, dilute BisU-converted DNA in a standard volume (e.g., 20  $\mu$ L) and set up a BisU DNA repair reaction master mix by pipetting BisU DNA Repair Buffer, 5x RNase-free Water, and BisU DNA Repair Enzyme Mix. Scale up for the number of required reactions and then add 10%.

**Table 3. BisU DNA repair reaction setup**

Component	Volume/reaction
BisU DNA repair Buffer, 5x	10 $\mu$ L
RNase-free Water	Variable
BisU DNA repair Enzyme Mix	2 $\mu$ L
BisU-converted DNA	Variable
<b>Total reaction volume</b>	<b>50 <math>\mu</math>L</b>

4. Add diluted BisU DNA to each BisU DNA Repair Reaction Mix on ice to a total volume reaction of 50  $\mu$ L and gently vortex to mix.
5. Briefly spin down the tubes, immediately transfer to the thermocycler, and start the programmed BisU DNA repair cycling program (Table 1) to allow double-strand synthesis and end-polishing of the fragments.
6. Place samples on ice after the completion of thermal cycling.

## Adapter ligation

7. During BisU DNA repair cycling, vortex and spin down the Adapter Plate. Pierce the plate foil seal and dilute adapters in RNase-free Water (Table 4).
8. For adapter ligation, use the dilutions in Table 4 depending on the DNA input in the complete workflow, including EpiTect Fast bisulfite conversion.

**Table 4. Adapter dilution**

DNA input in complete workflow	Adapter dilution in RNase-free Water
<500 pg	1:50
500 pg – 50 ng	1:10
>50 ng	1:2
>250 ng	1:1

9. Transfer 4  $\mu\text{L}$  of one adapter to each sample. Track the bar codes used and freeze the remaining adapters.
10. Prepare the ligation master mix on ice according to Table 5. Mix by gently vortexing and spin down shortly to collect liquids on the bottom of the tube.

**Table 5. Ligation master mix setup**

Component	Volume/reaction
Ultralow Input Ligation Buffer, 4X	25 $\mu\text{L}$
Ultralow Input Ligase	5 $\mu\text{L}$
RNase-free Water	16 $\mu\text{L}$
<b>Total reaction volume</b>	<b>46 <math>\mu\text{L}</math></b>

**Note:** Scale up for the number of required reactions, then add 10%.

11. Add 46  $\mu\text{L}$  ligation master mix to each sample to generate a total volume of 100  $\mu\text{L}$ . Vortex to mix, then spin down and place in the thermocycler.
12. Run the programmed ligation cycling program (Table 2). After completion of ligation step proceed immediately to the purification of the generated library.

## Library purification using QIAseq Beads

13. Add 50  $\mu\text{L}$  (0.5x) mixed QIAseq Beads to each sample and vortex.  
**Note:** equilibrate QIAseq Beads at room temperature before use.  
**Note:** If working with plates we recommend mixing by pipetting to avoid contamination during opening the sealing tapes. Always change pipette tips after each mixing step.
14. Incubate for 5 min at room temperature to bind library fragments on the beads.
15. Pellet the beads on a magnetic stand until supernatant is clear (approximately 5 min) and carefully discard the supernatant.
16. Add 200  $\mu\text{L}$  fresh 80% ethanol to each pellet to wash the beads immobilized on the magnet.
17. Discard the supernatant and repeat the washing step 16. After repeating the washing step, discard the supernatant. Carefully remove all remaining ethanol droplets from the tube inner walls since remaining ethanol contamination may inhibit subsequent reactions.
18. Incubate on the magnetic stand for 5–10 min or until the beads are dry (visual control). Avoid over-drying since this may result in lower DNA recovery. Remove from the magnetic stand.
19. Elute by resuspending the beads in 55  $\mu\text{L}$  RNase-free Water. Pellet beads on the magnetic stand until the supernatant is clear (approximately 5 min).
20. Carefully transfer 50  $\mu\text{L}$  supernatant to a new tube and proceed with the second purification, below.
21. Add 60  $\mu\text{L}$  (1.2x) QIAseq Beads to each sample, mix, and follow steps 14–18.
22. Elute by resuspending beads in 25  $\mu\text{L}$  RNase-free Water. Pellet the beads on the magnetic stand until supernatant is clear (approximately 5 min) and transfer 20  $\mu\text{L}$  supernatant into a new LoBind tube. The purified library can be stored at  $-20^{\circ}\text{C}$ .

# Protocol: Library Amplification

This procedure describes the NGS library amplification using the VeraSeq Ultra DNA Polymerase.

1. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 6.

**Note:** Cycle number of library amplification will vary depending on the starting material and should be estimated for each individual experiment. Table 7 provides some recommendations for the number of cycles that should be used for different input amounts in the complete workflow, starting with EpiTect Fast bisulfite conversion of DNA.

**Table 6. Library amplification cycling conditions**

Step	Temperature	Incubation Time
1	98°C	30 s
2 (6–18 cycles)	98°C	5 s
	55°C	10 s
	72°C	15 s
3	72°C	5 min
4	4°C	Hold

**Note:** Use a thermocycler with heated lid on.

**Table 7. Recommended PCR cycle numbers for library amplification**

DNA input in complete workflow	Number of PCR cycles
<500 pg	16–18
500 pg – 1 ng	14
1 ng – 50 ng	10
>50 ng	6
>250 ng	4

2. Thaw library DNA from step 22 of “Protocol: Library Generation from Bisulfite-Converted DNA”, 5X VeraSeq Buffer II, RNase-free Water, dNTP Mix, and Illumina primer mix and place on ice after thawing. Place VeraSeq Ultra DNA Polymerase directly onto ice. Mix thoroughly before use. Prepare reaction mix on ice.
3. Prepare the amplification reaction mix according to Table 8. Vortex to mix and spin down to collect liquid in the bottom of the tube.

**Table 8. Library amplification reaction mix**

<b>Component</b>	<b>Volume/reaction</b>
RNase-free Water	17.25 $\mu$ L
5X VeraSeq Buffer II	10 $\mu$ L
dNTP Mix	1.25 $\mu$ L
Illumina primer mix	1 $\mu$ L
VeraSeq Ultra DNA Polymerase	0.5 $\mu$ L
<b>Total</b>	<b>30 <math>\mu</math>L</b>

**Note:** Scale up for the number of required reactions, then add 10%.

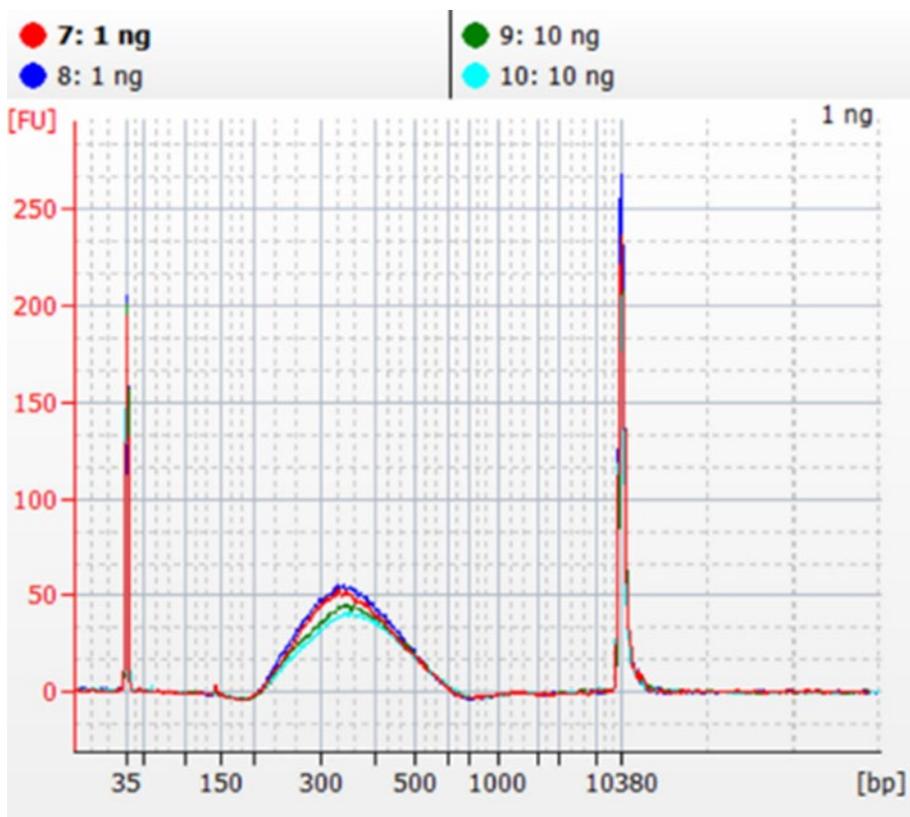
4. Add 30  $\mu$ L amplification reaction mix to 20  $\mu$ L library (from step 22 of “Protocol: Library Generation from Bisulfite-Converted DNA”), vortex to mix, then spin down and place on ice.
5. Place the PCR tubes in the thermal cycler and start the programmed library amplification cycling program (Table 6).
6. After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

## Final library purification using QIAseq Beads

7. Add 55  $\mu\text{L}$  (1.1x volume) QIAseq Beads to 50  $\mu\text{L}$  amplified library and mix well by pipetting up and down.
8. Incubate for 5 min at room temperature to bind library fragments on the beads.
9. Pellet the beads on a magnetic stand until supernatant is clear (approximately 5 min) and carefully discard the supernatant.
10. Add 200  $\mu\text{L}$  fresh 80% ethanol to each pellet to wash the beads immobilized on magnet.
11. Discard the supernatant and repeat the washing step 10.
12. After repeating the washing step, discard the supernatant. Carefully remove all remaining ethanol droplets from the tube inner walls, since remaining ethanol contamination may inhibit subsequent reactions.
13. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Avoid over-drying since this may result in lower DNA recovery. Remove from the magnetic stand.
14. Elute by resuspending and incubating beads for 5 min at room temperature in 25  $\mu\text{L}$  RNase-free Water.
15. Pellet the beads on the magnetic stand for 5 min and transfer 20  $\mu\text{L}$  supernatant into a new LoBind tube. Store at  $-20^{\circ}\text{C}$  until sequencing.
16. Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the correct size distribution (Figure 3) of library fragments and for the absence of adapters or adapter–dimers.

**Note:** the median size of the DNA inserts is approximately 250 bp and should be shifted by the size of the adapters 120 bp that were ligated to the library fragments.

**Note:** the median fragment size can be used for subsequent qPCR-based quantification methods.



**Figure 3. Capillary electrophoresis device trace of generated libraries.** Capillary electrophoresis device trace data showing the correct size distribution of library fragment and the absence of adapters or adapter-dimers. Shown are replicate libraries starting from 1 ng or 10 ng bisulfite-converted DNA. Library of 1 ng was amplified for 14 cycles and 10 ng library was amplified using 10 cycles of PCR. Libraries were diluted 1:10 and loaded on an Agilent High Sensitivity DNA chip.

## Reference

1. Ziller, M. J., Hansen, K. D., Meissner, A., & Aryee, M. J. (2015). Coverage recommendations for methylation analysis by whole-genome bisulfite sequencing. *Nature methods*, *12*(3), 230-232.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: [www.qiagen.com/FAQ/FAQList.aspx](http://www.qiagen.com/FAQ/FAQList.aspx). The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit [www.qiagen.com](http://www.qiagen.com)).

## Comments and suggestions

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### Low library yields from library preparation protocol

- |  |   |
|--|---|
| a) Bisulfite-converted DNA yields were lower as expected                           | Quantify the yield of bisulfite-converted DNA using NanoDrop or Agilent RNA chips. Please note that very low amounts will not be quantified accurately.<br>EpiTect Fast bisulfite conversion, column desulfonation, and purification lead to 30–50% DNA loss depending on the input amount and quality of starting material. Take that into consideration while designing the experiment and adjust input for optimal result.   |
| b) Suboptimal reaction conditions due to low DNA quality                           | DNA fragmentation was too high due to low-integrity DNA input. Adjust denaturation time during the EpiTect Fast protocol.   |
| c) Suboptimal reaction conditions due to ethanol contamination                     | Ensure that ethanol residue has been fully removed during EpiTect washing steps and residual liquid removal, since ethanol contamination inhibits the subsequent enzymatic reactions.   |
| d) Suboptimal reaction conditions during double-strand synthesis and end-polishing | Ensure that cycling conditions and incubation temperatures are programmed correctly.  |
| e) Inefficient library amplification   | Inefficient adapter–dimer removal using QIAseq Beads. Mix beads thoroughly and use the recommended amount for purification.<br>Beads are overdried. Make visual control during drying of beads; humidity of environment may accelerate or slow the drying procedure.<br>Ethanol residues inhibit the reaction. Ensure that all liquids have been removed prior to beads drying. If uncertain, perform a short spin to collect the liquid droplets and remove carefully before drying the beads. |

## Comments and suggestions

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### Unexpected signal peaks in capillary electrophoresis device traces

- |  |  |
|--|--|
| a) Presence of shorter peaks between 60 bp and 120 bp            | These peaks represent library adapters and adapter–dimers that occur when there is no – or insufficient – adapter depletion after library preparation. As adapter–dimers can form clusters on the flow cell and will be sequenced, this will reduce the capacity of the flow cell for the library fragments, even though a low ratio of adapter–dimers versus library will not be a problem. QIAseq Beads efficiently remove adapter–dimers, as well as free adapter molecules.  |
| b) Presence of larger library fragments after library enrichment | When performing library enrichment, if the fragment population shifts higher than expected after adapter ligation and PCR enrichment (e.g., more than the expected 120 bp shift), this may be a PCR artifact due to over-amplification of the DNA library. Reduce the number of amplification cycles.  |
| c) Incorrect library fragment size after adapter ligation        | During library preparation, adapters of approximately 60 bp are ligated to both ends of the DNA library fragments. This should be reflected on a capillary electrophoresis device by a shift in size of all library fragments of 120 bp. If using library adapters from other suppliers, please refer to the size information given in the respective documentation. The absence of a clear size shift may indicate no – or only low – adapter ligation efficiency. Be sure to use the parameters and incubation times described in the handbook for end-repair, A-addition and ligation, as well as the correct starting amount of DNA. |

# Appendix A: Sequencing Recommendations on Illumina Platforms

Table 9 lists recommended library concentration levels for optimal clustering using MiSeq chemistries Version v2 or v3 with libraries generated from gDNA, ccfDNA, or prefragmented DNA.

**Table 9. Optimal library concentration**

DNA	Molarity	Raw density (K/mm <sup>2</sup> )
Bisulfite-converted gDNA	15–17.5 pM	1000–1200
Bisulfite-converted FFPE DNA	15–17.5 pM	1000–1200
ccfDNA	10 pM	1000–1200
Prefragmented DNA (approx. 200 bp)	10 pM	1000–1200

For all other instruments please follow the instructions described in ILLUMINA “Denature and Dilute Libraries Guide” of each individual instrument.

For bisulfite sequencing it is recommended to add PhiX Control library during sequencing in order to increase base complexity and increase the sequencing quality. We recommend using 10% of PhiX Control v3 Library during sequencing. For instructions on how to denature and dilute PhiX Control please consult ILLUMINA “Denature and Dilute Libraries Guide” of each individual instrument.

Due to fragmentation of DNA during bisulfite treatment and the nature of library generation based on ligation of adapters it is recommended to keep sequencing length short to avoid sequencing over the adapter sequences and an increased number of chimeric reads. Table 10 provides sequencing recommendations depending on the sequencing instrument and type analysis.

**Table 10. Sequencing recommendations for methylation sequencing**

Instrument	Sequencing recommendation for QC of library pool before deeper sequencing	Sequencing recommendation for WGBS/RRBS
MiSeq (MiSeq Reagent Nano Kit v2)	PE 2 x 150 bp	–
NextSeq 500	–	PE 2 x 75 bp
HiSeq 1500/2500	–	PE 2 x 50 bp PE 2 x 100 bp PE 2 x 125 bp
NovaSeq	–	PE 2 x 50 bp PE 2 x 100 bp

For genome coverage recommendations please refer to *Coverage recommendations for methylation analysis by whole genome bisulfite sequencing* by Ziller et al. (1).

For Whole Genome Bisulfite Sequencing (WGBS) the recommended sequencing depth to achieve approximately 30x of coverage is 90–120 Gb. The Reduced Representation Bisulfite Sequencing (RRBS) requires 3–5 Gb per sample in order to capture 10–15% of CpG and ~75% of CpG islands with an average coverage of 30x.

Table 11 summarizes the recommendations for WGBS library multiplexing on Illumina sequencing instruments with sequencing length 2x 100 bp to yield an average coverage of 15x–30x.

**Table 11. Library multiplexing recommendations**

Instrument	Chemistry	Number of WGBS libraries to achieve approx. average coverage 15X	Number of WGBS libraries to achieve approx. average coverage 30X
NextSeq 500	High Output kit	–	1 per flow cell
HiSeq 1500/2500	High Output v4 Reagents	1 per lane	0.5 per lane
NovaSeq	SP Reagent Kit	3 per flow cell	1 per flow cell
NovaSeq	S1 Reagent Kit	5 per flow cell	3 per flow cell
NovaSeq	S2 Reagent Kit	14 per flow cell	7 per flow cell
NovaSeq	S4 Reagent Kit	33 per flow cell	17 per flow cell

# Appendix B: QIAseq Dual-Index Y-Adapters

## Generation of sample sheets for Illumina instruments

Index sequences for QIAseq Unique and Combinatorial Dual-Index Y-Adapters are available for download at [www.qiagen.com/PROM-17090](http://www.qiagen.com/PROM-17090).

1. Create a run using Illumina Local Run Manager by selecting **+ Create Run** and **Generate FASTQ**.
2. Enter the information of the Run Name and Description and paste the index sequence in the Sample and Indexes table. Add as many rows in the table as indexes have been used.
3. Save the run or export the Sample Sheet.

## Unique Dual-Index Y-Adapters

The layout of the 24-plex single-use UDI adapter plate is shown in Figure 4. The index motifs used in the QIAseq Unique Dual-Index Kits are listed in Table 12. To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments at [www.qiagen.com](http://www.qiagen.com).

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	UDI 001	UDI 009	UDI 017	empty								
<b>B</b>	UDI 002	UDI 010	UDI 018	empty								
<b>C</b>	UDI 003	UDI 011	UDI 019	empty								
<b>D</b>	UDI 004	UDI 012	UDI 020	empty								
<b>E</b>	UDI 005	UDI 013	UDI 021	empty								
<b>F</b>	UDI 006	UDI 014	UDI 022	empty								
<b>G</b>	UDI 007	UDI 015	UDI 023	empty								
<b>H</b>	UDI 008	UDI 016	UDI 024	empty								

**Figure 4. QIAseq UDI Y-Adapter Plate (24) layout (UDI 1–24).**

**Table 12. UDI motifs used in the QIAseq UDI Y-Adapter Kits (24)**

Indices for entry on sample sheet

Unique dual-index number	i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/2500, NovaSeq 6000 v1.0)	i5 bases for entry on sample sheet (iSeq100, MiniSeq, NextSeq, HiSeqX, HiSeq 3000/4000, NovaSeq 6000 v1.5)	i7 bases for entry on sample sheet (all instruments)
UDI 001	ATGGCCGACT	AGTCGGCCAT	TGAACGTTGT
UDI 002	CGATGAGCAC	GTGCTCATCG	ACCAGACTTG
UDI 003	GATAAGTCGA	TCGACTTATC	ACTGGCGAAC
UDI 004	TCACGCCTTG	CAAGGCGTGA	GCGTTAGGCA
UDI 005	AGGAACACAA	TTGTGTTCTT	TTATCGGCCT
UDI 006	CTCAGTAGGC	GCCTACTGAG	GAGGTATAAG
UDI 007	GAAGTGCCTG	CAGGCACTTC	TCAAGGATTC
UDI 008	TCTCTCGCCT	AGGCGAGAGA	CGAACCGAGA
UDI 009	AGGCACCTTC	GAAGGTGCCT	GAGCCAAGTT
UDI 010	CTGTTGGTAA	TTACCAACAG	AAGGCCGTAG
UDI 011	GCTGGTACCT	AGGTACCAGC	TTAGAGAAGC
UDI 012	TAAGGAGCGG	CCGCTCCTTA	TCTAAGACCA
UDI 013	AATCGCTCCA	TGGAGCGATT	TGTAACCACT
UDI 014	CTCCTAATTG	CAATTAGGAG	CCGACACAAG
UDI 015	GCCTCATAAT	ATTATGAGGC	CTCTGATGGC
UDI 016	TGTATTGAGC	GCTCAATACA	CGGCCTGTTA
UDI 017	AGCCATAACA	TGTTATGGCT	TGCATAGCTT
UDI 018	CCACAAGTGG	CCACTTGTGG	AACCTTCTCG
UDI 019	GTTATCACAC	GTGTGATAAC	AAGAGATCAC
UDI 020	TACCGTTCTT	AAGAACGGTA	GCCTGAAGGA
UDI 021	AGGCGTTAGG	CCTAACGCCT	ATTGTGCCTT
UDI 022	CCGTAACGTC	GACGTTACGG	TCCTTACCG
UDI 023	GTAATAGCCA	TGGCTATTAC	TACCATGAAC
UDI 024	TAGCGCCGAT	ATCGGCGCTA	CATTGGCAGA

Unique Dual-Index adapters 1–24 are identical to the adapter plates of the QIAseq UDI Y-Adapter Kit (24) and QIAseq UDI Y-Adapter Kit A (96).

**Note:** Sequencing on the MiniSeq, NextSeq, HiSeqX, and HiSeq 3000/4000 systems follows a dual-indexing workflow different from other Illumina systems, which requires the reverse complement of the i5 index adapter sequence.

# Appendix C: Bisulfite Conversion of Purified gDNA and FFPE DNA Using EpiTect Fast DNA Bisulfite Conversion Kits

## Bisulfite conversion of DNA

This protocol describes the procedure of bisulfite treatment of DNA and FFPE using the EpiTect Fast Bisulfite Conversion Kits. We recommend to carefully read the EpiTect Fast Handbook for more details, applications, and cautions for reagent handling.

**Note:** the EpiTect Fast Bisulfite Conversion Kit must be acquired separately.

1. Thaw DNA to be used in the bisulfite reactions. Make sure the Bisulfite Solution is completely dissolved and equilibrated to room temperature.

**Note:** if necessary, heat the Bisulfite Solution to 60°C and vortex until all precipitates are dissolved again.

**Note:** do not place dissolved Bisulfite Solution on ice.

**Important:** We recommend not to use carrier RNA to avoid introduction of any contamination. However, in case that sample is very limited (<1 ng), addition of carrier RNA during bisulfite treatment will increase recovery.

2. Prepare the bisulfite reactions in 200 µL PCR tubes (not supplied) according to Table 13. Add each component in the order listed.

**Table 13. Preparation of bisulfite reactions**

Component	Volume/reaction
DNA (10 pg – 500 ng)*	Variable (maximum 55 µL)
RNase-free Water	Variable
Bisulfite Solution	85 µL
<b>Total reaction volume</b>	<b>140 µL</b>

\* Optimal: 1 ng – 100 ng. Consider that 30–50% of DNA will be lost during bisulfite conversion. Input lower than 500 pg will lead to reduced mapping.

- Close the PCR tubes and mix the bisulfite reactions thoroughly. Store the tubes at room temperature (15–25°C).
- Perform the bisulfite DNA conversion using a thermal cycler according to Table 14. The complete cycle should take approximately 80 min.

**Note:** if using a thermal cycler that does not allow you to enter the reaction volume (140 µL), set the instrument to the largest volume setting available.

**Important:** if using strongly digested DNA, for example DNA purified from FFPE material, we recommend reducing denaturation time to 5 min and bisulfite conversion incubation time to 15 min per cycle.

**Table 14. Bisulfite conversion cycling conditions**

Step	Temperature	Incubation time gDNA	Incubation time FFPE DNA
Denaturation	95°C	10 min	8 min
Incubation	60°C	30 min	30 min
Denaturation	95°C	10 min	8 min
Incubation	60°C	30 min	30 min
Hold	20°C	Indefinite	Indefinite

## Cleanup of bisulfite-converted DNA

5. Once the bisulfite conversion is complete, briefly centrifuge the PCR tubes containing the bisulfite reactions, and then transfer the complete bisulfite reactions to clean 1.5 mL microcentrifuge tubes.

Transfer of precipitates in the solution will not affect the performance or yield of the reaction.

6. Add 310  $\mu\text{L}$  freshly prepared Buffer BL to each sample. Mix the solutions by vortexing and then centrifuge briefly.
7. Add 250  $\mu\text{L}$  ethanol (96–100%) to each sample. Mix the solutions by pulse vortexing for 15 s, and centrifuge briefly to remove the drops from inside the lid.
8. Place the necessary number of MinElute<sup>®</sup> DNA spin columns and collection tubes in a suitable rack. Transfer the entire mixture from each tube from step 7 into the corresponding MinElute DNA spin column.
9. Centrifuge the spin columns at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
10. Add 500  $\mu\text{L}$  Buffer BW (wash buffer) to each spin column, and centrifuge at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
11. Add 500  $\mu\text{L}$  Buffer BD (desulfonation buffer) to each spin column then close the lids of the spin columns and incubate for 15 min at room temperature (15–25°C).  
If there are precipitates in Buffer BD, avoid transferring them to the spin columns.  
**Important:** the bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in air.
12. Centrifuge the spin columns at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
13. Add 500  $\mu\text{L}$  Buffer BW to each spin column and centrifuge at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
14. Repeat step 13 once.

15. Add 250  $\mu\text{L}$  ethanol (96–100%) to each spin column and centrifuge at maximum speed for 1 min.
16. Place the spin columns into new 2 mL collection tubes and centrifuge the spin columns at maximum speed for 1 min to remove any residual liquid.  
**Optional:** Place the spin columns with open lids into a clean 1.5 mL microcentrifuge tube (not provided) and incubate the columns for 5 min at 60°C in a heating block. This step ensures the evaporation of any remaining liquid and removal of any ethanol residual contamination.
17. Place the spin columns into clean 1.5 mL microcentrifuge tubes (not provided). Add 18  $\mu\text{L}$  RNase-free Water directly onto the center of each spin-column membrane and close the lids gently.
18. Incubate the spin columns at room temperature for 1 min.
19. Centrifuge for 1 min at 15,000  $\times g$  (12,000 rpm) to elute the DNA.

**Note:** We recommend storing purified DNA at 2–8°C for up to 24 h. When storing purified DNA for longer than 24 h, we recommend storage at –20°C. Avoid storing single-stranded, bisulfite-converted DNA for longer than 2–3 weeks.

**Note:** In this protocol and during bisulfite treatment, in absence of DNA protect, DNA is being fragmented due to repeated heat application and can be used without additional fragmentation for the library preparation. However, for standardization purposes or enrichment of specific regions such as CpG Island, DNA may be preferred to be fragmented prior to library preparation. In such cases, please follow the bisulfite conversion protocol described in Appendix D, page 36 in this Handbook.

# Appendix D: Bisulfite Conversion of Prefragmented (~200 bp) and ccfDNA Using EpiTect Fast Bisulfite Conversion Kits

This protocol can be used with purified ccfDNA, for example using the QIAamp® MinElute ccfDNA Kit or QIAamp MinElute ccfDNA Midi Kit, and DNA that has been previously fragmented and/or undergone enrichment of CpG islands.

This protocol describes the procedure of bisulfite treatment of ccfDNA and fragmented DNA using the EpiTect Fast Bisulfite Conversion Kits. We recommend to carefully read the EpiTect Fast Handbook to more details, applications, and cautions for reagent handling.

**Note:** the EpiTect Fast Bisulfite Conversion Kit has to be acquired separately.

## Bisulfite conversion of DNA

1. Thaw DNA to be used in the bisulfite reactions. Make sure the Bisulfite Solution is completely dissolved and equilibrated to room temperature.

**Note:** if necessary, heat the Bisulfite Solution to 60°C and vortex until all precipitates are dissolved again.

**Note:** do not place dissolved Bisulfite Solution on ice.

**Important:** We recommend not to use carrier RNA to avoid introduction of any contamination. However, in case of very limited sample (<1 ng), addition of carrier RNA during bisulfite treatment will increase recovery.

2. Prepare the bisulfite reactions in 200 µL PCR tubes (not supplied) according to Table 15. Add each component in the order listed.

**Table 15. Preparation of bisulfite reactions**

Component	Volume/reaction
DNA (10 pg – 500 ng)*	Variable (maximum 40 µL)
RNase-free Water	Variable
Bisulfite Solution	85 µL
DNA Protect Buffer	15 µL
<b>Total reaction volume</b>	<b>140 µL</b>

\* Optimal: 1 ng – 100 ng. Consider that 30-50% of DNA will be lost during bisulfite conversion. Input lower than 500 pg will lead to reduced mapping. DNA Protect can be increased up to 35 µL if DNA concentration is high enough to allow this volume. Final volume should not exceed 140 µL.

3. Close the PCR tubes and mix the bisulfite reactions thoroughly. Store the tubes at room temperature (15–25°C).

**Note:** DNA Protect Buffer should turn from green to blue after addition to the DNA–Bisulfite Solution mixture, indicating sufficient mixing and correct pH for the bisulfite conversion reaction.

4. Perform the bisulfite DNA conversion using a thermal cycler according to the following cycling conditions (Table 16). The complete cycle should take approximately 30 min.

**Note:** if using a thermal cycler that does not allow you to enter the reaction volume (140 µL), set the instrument to the largest volume setting available.

**Table 16. Bisulfite conversion cycling conditions**

Step	Temperature	Incubation Time
Denaturation	95°C	5 min
Incubation	60°C	10 min
Denaturation	95°C	5 min
Incubation	60°C	10 min
Hold	20°C	Indefinite

## Cleanup of bisulfite-converted DNA

5. Once the bisulfite conversion is complete, briefly centrifuge the PCR tubes containing the bisulfite reactions, and then transfer the complete bisulfite reactions to clean 1.5 mL microcentrifuge tubes.

Transfer of precipitates in the solution will not affect the performance or yield of the reaction.

6. Add 310  $\mu\text{L}$  freshly prepared Buffer BL to each sample. Mix the solutions by vortexing and then centrifuge briefly.
7. Add 250  $\mu\text{L}$  ethanol (96–100%) to each sample. Mix the solutions by pulse vortexing for 15 s, and centrifuge briefly to remove the drops from inside the lid.
8. Place the necessary number of MinElute DNA spin columns and collection tubes in a suitable rack. Transfer the entire mixture from each tube from step 7 into the corresponding MinElute DNA spin column.
9. Centrifuge the spin columns at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
10. Add 500  $\mu\text{L}$  Buffer BW (wash buffer) to each spin column, and centrifuge at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
11. Add 500  $\mu\text{L}$  Buffer BD (desulfonation buffer) to each spin column then close the lids of the spin columns and incubate for 15 min at room temperature (15–25°C).  
If there are precipitates in Buffer BD, avoid transferring them to the spin columns.  
**Important:** the bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in air.
12. Centrifuge the spin columns at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
13. Add 500  $\mu\text{L}$  Buffer BW to each spin column and centrifuge at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
14. Repeat step 13 once.

15. Add 250  $\mu\text{L}$  ethanol (96–100%) to each spin column and centrifuge at maximum speed for 1 min.
16. Place the spin columns into new 2 mL collection tubes and centrifuge the spin columns at maximum speed for 1 min to remove any residual liquid.  
**Optional:** Place the spin columns with open lids into a clean 1.5 mL microcentrifuge tube (not provided) and incubate the columns for 5 min at 60°C in a heating block. This step ensures the evaporation of any remaining liquid.
17. Place the spin columns into clean 1.5 mL microcentrifuge tubes (not provided). Add 18  $\mu\text{L}$  RNase-free Water directly onto the center of each spin-column membrane and close the lids gently.
18. Incubate the spin columns at room temperature for 1 min.
19. Centrifuge for 1 min at 15,000  $\times g$  (12,000 rpm) to elute the DNA.  
**Note:** We recommend storing purified DNA at 2–8°C for up to 24 h. When storing purified DNA for longer than 24 h, we recommend storage at –20°C.  
**Note:** during bisulfite treatment DNA is fragmented and can be used without additional fragmentation for the library preparation.

# Ordering Information

Product	Contents	Cat. no.
QIAseq Methyl Library Kit (24)	Enzymes, Buffers, Reagents for 24 double-strand synthesis and end-polishing of bisulfite-converted DNA, ligation of Illumina adapters, library purification and amplification. Generated libraries are for use with Illumina Instruments	180502
<b>Related products</b>		
QIAseq CDI Y-Adapter Kit (24)	1 single-use adapter plate containing differently barcoded CDI Y-adapters for 24 reactions; pierceable foil seal allowing usage of defined parts of the plate	1118255
QIAseq CDI Y-Adapter Kit (96)	1 single-use adapter plate containing differently barcoded CDI Y-adapters for 96 reactions; pierceable foil seal allowing usage of defined parts of the plate	1118254
QIAseq UDI Y-Adapter Kit A (96)	1 single-use adapter plate containing differently barcoded UDI Y-adapters for 96 reactions (UDI 1–96); pierceable foil seal allowing usage of defined parts of the plate	1118257
QIAseq UDI Y-Adapter Kit B (96)	1 single-use adapter plate containing differently barcoded UDI Y-adapters for 96 reactions (UDI 97–192); pierceable foil seal allowing usage of defined parts of the plate	1118258
QIAseq UDI Y-Adapter Kit C (96)	1 single-use adapter plate containing differently barcoded UDI Y-adapters for 96 reactions (UDI 193–288); pierceable foil seal allowing usage of defined parts of the plate	1118259
QIAseq UDI Y-Adapter Kit D (96)	1 single-use adapter plate containing differently barcoded UDI Y-adapters for 96 reactions (UDI 289–384); pierceable foil seal allowing usage of defined parts of the plate	1118260

Product	Contents	Cat. no.
QIAseq Library Quant Assay Kit	Laboratory-verified forward and reverse primers for 500 x 25 µL reactions (500 µL); DNA Standard (100 µL); Dilution Buffer (30 mL); (1.35 mL x 5) GeneRead™ qPCR SYBR® Green Mastermix	333314
EpiTect Fast Bisulfite Kit (10)	Trial kit for 10 preps: Deparaffinization Solution, Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA, and Buffers	59802
EpiTect Fast DNA Bisulfite Kit (50)	For 50 preps: Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA, and Buffers	59824
EpiTect Fast LyseAll Bisulfite Kit (50)	For 50 preps: Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA, and Buffers	59864
QIAamp MinElute ccfDNA Mini Kit (50)	For 50 preps (1 or 2 mL sample input volume each): QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, Bead Elution Tubes, Collection Tubes (1.5 mL and 2 mL)	55204
QIAamp MinElute ccfDNA Midi Kit (50)	For 50 preps (4 or 5 mL sample input volume each): QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, Bead Elution Tubes, and Collection Tubes (1.5 mL and 2 mL)	55284

\* Other kit sizes/formats available; see [www.qiagen.com](http://www.qiagen.com).

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# Document Revision History

Revision	Description
November 2017	Initial release
July 2022	Several sections were added and/or adapted to the inclusion of QIAseq UDI Y-Adapter Kit. Added Illumina NovaSeq to the list of compatible sequencing platforms. Updated the list of equipment and reagents to be supplied by user, Tables 4 and 7, and Figure 4. Appendix A now includes sequencing recommendations for Illumina Platforms. Revised Appendices C and D to discuss protocols using the EpiTect Fast Bisulfite Conversion Kits.

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