Quick-Start Protocol October 2017

QIAseq™ Methyl DNA Library Kit

The QIAseq Methyl DNA Library Kit (cat. no. 180503) should be stored immediately upon receipt at –15°C to –30°C and QIAseq Beads (cat. no. 1107921) at 2–8°C. If stored under these conditions, kits are stable until the date indicated on the QC label inside the kit lid.

Further information

- *QIAseq Methyl DNA Library Handbook*: **www.qiagen.com/HB-2476**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for construction of whole genome bisulfite sequencing libraries for Illumina® NGS platforms starting from single-stranded bisulfite-converted DNA. Detailed protocols and recommendations are included in the handbook.

Procedure: Library generation from bisulfite-converted DNA

This procedure describes the NGS library preparation of the bisulfite-converted, single-stranded and fragmented DNA and include the BisU DNA repair reaction, adapter ligation and library amplification.

1. Program a thermal cycler with the protocols described in Table 1 and Table 2.

Table 1. BisU DNA repair cycling conditions

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

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

Table 2. Ligation cycling conditions

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

Note: Use a thermocycler with heated lid off.

2. Setup the BisU DNA repair reaction mix on ice according to Table 3 and mix by gently vortexing. Keep reaction on ice.

Table 3. BisU DNA repair reaction mix setup

Component	Volume/reaction
BisU DNA repair Buffer, 5x	10 µl
RNase-Free H_2O	Variable
BisU DNA repair Enzyme Mix	2 µl
BisU-converted DNA	Variable
Total reaction volume	**50 µl**

3. Transfer to the thermocycler and start the BisU DNA repair cycling program (Table 1). Place samples on ice, after cycling completion.
4. During BisU DNA repair cycling, prepare adapter dilutions in RNase-Free H_2O as recommended in the *QIAseq Methyl DNA Library Handbook* and transfer 4 µl of one adapter to each sample. Track the bar codes used.

5. Prepare the ligation master mix on ice according to Table 4 and mix gently.

Table 4. Ligation master mix setup

Component	Volume/reaction
Ultralow Input Ligation Buffer, 4X	25 µl
Ultralow Input Ligase	5 µl
RNase-Free H_2O	16 µl
Total reaction volume	**46 µl**

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

6. Add 46 µl ligation master mix to each sample. Mix by short vortexing, spin down and place in the thermocycler. Run the ligation cycling program (Table 2).
7. For library purification, mix 50 µl (0.5x) QIAseq Beads with each sample.
8. Incubate for 5 min at room temperature. Immobilize beads and discard the clear supernatant.
9. Add 200 µl fresh 80% ethanol to each beads pellet immobilized on the magnet.
10. Discard the supernatant, repeat washing step 9 and discard the supernatant.
11. Incubate on the magnetic stand for 5–10 min, until the beads are dry. Over-drying may result in lower DNA recovery. Remove from the magnetic stand.
12. Elute by resuspending in 55 µl RNase-Free H_2O. Immobilize beads and transfer 50 µl supernatant to a new tube.
13. Mix 60 µl (1.2x) QIAseq beads with each sample and repeat steps 8–11.
14. Elute by resuspending beads in 25 µl RNase-Free H_2O. Immobilize the beads and transfer 20 µl of supernatant into a new tube. Store at –15 to –30°C.
15. For library amplification thaw library DNA from step 14, and library amplification reagents on ice.
16. Prepare a reaction mix according to Table 5 and mix with 20 µl library (step15).

Table 5. Library amplification reaction mix

Component	Volume/reaction
RNase-Free H_2O	17.25 µl
5X VeraSeq Buffer II	10 µl
dNTP Mix	1.25 µl
Illumina primer mix	1 µl
VeraSeq Ultra DNA Polymerase	0.5 µl
Total	**30 µl**

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

17. Place the PCR tubes in the thermal cycler and start the pre-programmed library amplification cycling program with the conditions outlined in Table 6.

Table 6. Library amplification cycling conditions

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

Note: Use a thermocycler with heated lid on. For number of cycles, consult the *QIAseq Methyl DNA Library Handbook*.

18. Purify library using QIAseq Beads and store as described in the Handbook.

Scan QR code for handbook.