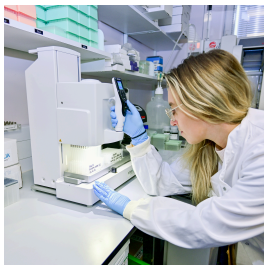
Dec 09, 2025

DNA Bisulfite conversion, using Zymo EZ-96 DNA Methylation-Lightning MagPrep Kit and Integra VIAFLO 96, for processing on Illumina methylation arrays



DOI
https://dx.doi.org/10.17504/protocols.io.81wgbkj9qgpk/v1

Emma Aitken[1], Nicola Wrobel[1], Lee Murphy[1]

[1]Genetics Core, Edinburgh Clinical Research Facility, University of Edinburgh

Lee Murphy
Genetics Core, Edinburgh Clinical Research Facility, Univers...

DOI: https://dx.doi.org/10.17504/protocols.io.81wgbkj9qgpk/v1

Protocol Citation: Emma Aitken, Nicola Wrobel, Lee Murphy 2025. DNA Bisulfite conversion, using Zymo EZ-96 DNA Methylation-Lightning MagPrep Kit and Integra VIAFLO 96, for processing on Illumina methylation arrays. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbkj9qgpk/v1

Protocol status: Working
We use this protocol and it's working

Created: June 17, 2025

Last Modified: December 09, 2025

Protocol Integer ID: 220381

Keywords: dna methylation analysis service, throughput dna bisulfite conversion method, illumina methylation arrays the genetics core, dna bisulfite conversion, illumina methylation array, dna methylation, analysing dna sample, integra viaflo 96 platform

Abstract

The Genetics Core of the Edinburgh Clinical Research Facility provides DNA methylation analysis services using optimized automated protocols. This protocol details a high-throughput DNA bisulfite conversion method that uses Zymo EZ-96 DNA Methylation-Lightning MagPrep Kits and automated using the Integra VIAFLO 96 platform. The method is used as the first step in analysing DNA samples on Illumina Infinium Methylation Screening Arrays-48. This protocol allows 192 samples to be processed together.

Guidelines

This protocol details a high-throughput DNA bisulfite conversion method that uses Zymo EZ-96 DNA Methylation-Lightning MagPrep Kits and automated using the Integra VIAFLO 96 platform. The method is used as the first step in analysing DNA samples on Illumina Methylation Screening Arrays.

This protocol allows 2× 96-well plates (192 samples) to be processed together.

Materials

Zymo Research EZ-96 DNA Methylation-Lightning MagPrep Kit (D5046)

Integra VIAFLO 96 electronic pipette

Integra VIAFLO 96 Racks Sterile 300 µl (6434)

Integra VIAFLO 96 Racks Sterile Filter 300 µl (6435)

ThermoMixer e.g. Eppendorf ThermoMixer C (2231001127)

PCR Thermocycler e.g. Eppendorf Mastercycler X50 (2231001152)

Zymo Research ZR-96 Magnetic Stand (P1005)

Troubleshooting

Safety warnings

Step 19 is crucial. Samples need to be incubated for at least 15 mins and no longer than 25 mins. This limits the throughput of plates per operator.

Buffer Preparation

1 Add 288 mL of 95–100% ethanol to the 72 mL M-Wash Buffer concentrate. Mix well.

2 Label the buffer with preparation date and initials.

Sample Conversion

3 Transfer 20 µL of 50ng/ul (500ng) DNA into each well of the Conversion Plate.

4 Add 130 µL of Conversion Reagent (pre-mixed and light-sensitive). Mix thoroughly.

5 Seal with foil seal, centrifuge at 280 g for 30 seconds.

6 Place plate into the Eppendorf Mastercycler with settings:
98°C for 8 minutes
54°C for 60 minutes
Hold at 4°C (up to 20 hours)

Preparation of Collection Plate During Incubation

7 Add 600 µL of M-Binding Buffer and 10 µL of EZ-Methylation MagPrep Beads to each well of a fresh Collection Plate.

8 Keep beads suspended by gently vortexing or pipetting as needed.

Transfer to Collection Plate

9 Spin conversion plate at 280g for 30 seconds

10 Using VIAFLO 96 mix programme (aspirate 170ul at Asp speed = 1 and Mix speed = 8) and 300 µL **sterile filter** tips, transfer entire sample volume from Conversion Plate to

corresponding wells of Collection Plate.

11 In collection plate, use VIAFLO 96's mix function: mix 300 µL × 6 cycles to ensure bead binding. Dispose of tips.

12 Incubate at room temperature for 5 minutes.

Bead Separation

13 Place Collection Plate onto the ZR-96 Magnetic Stand for 5 minutes to pellet the beads.

14 Remove supernatant carefully using VIAFLO 96 at lowest speed setting. (Aspirate 300 µL at Asp speed = 1 and Disp. speed = 3) using 300 µL **sterile tips** (these do not need to be filter tips). Dispose tips

Wash Step

15 Remove plate from magnetic stand.

16 Add 400 µL of M-Wash Buffer, mix for 30 seconds at 1100 rpm on Eppendorf ThermoMixer C.

17 Return plate to magnetic stand for 3 minutes, remove supernatant.

Desulphonation

18 Add 200 µL of L-Desulphonation Buffer, mix on ThermoMixer for 30 seconds at 1100 rpm.

19 Incubate at room temperature for 15 minutes.

20 Place on magnetic stand for 3 minutes, remove supernatant.

21 Note: Take time for handling/re-suspension into account for the total incubation time. Adjust time as necessary to ensure that no sample remains in the L-Desulphonation Buffer for more than 20–25 minutes. This is particularly important when processing two plates together.

Final Washes and Elution

22 Add 400 µL of M-Wash Buffer, mix for 30 seconds at 1100 rpm, return to magnetic stand for 3 minutes, and discard supernatant.

23 Repeat the wash step once more (total: 2 washes).

24 Note: Remove as much buffer as possible after final wash to aid in the drying of the beads.

25 After second wash, dry beads by heating the plate at 55°C for 25 minutes on the ThermoMixer.

DNA Elution

26 Add 25 µL of Elution Buffer. Mix for 30 seconds at 1300 rpm on the ThermoMixer.

27 Heat the elution at 55ºC for 4 minutes then transfer the plate to the magnetic stand for 1 minute. Remove the supernatant and transfer to a clean Elution Plate using VIAFLO 96 pipette function (Aspirate 30ul at Asp speed = 1 and Disp. speed = 3) and 300 µL **sterile filter** tips.

28 Eluted DNA is now ready for downstream analysis or can be stored at -20°C.

Protocol references

Zymo EZ-96 DNA Methylation-Lightning MagPrep Kit Protocol Ver.2.1.1
(**d5046-e_d5047-e_ez-96_dna_methylation-lightning_magprep.pdf**)
Accessed 1 Dec 2025

Illumina Infinium EX Methylation Assay Semi-Automated Workflow
Document #200048699 v01
(https://support-docs.illumina.com/ARR/Inf-MSA-all/Content/ARR/FrontPages/MSA_all.htm)
Accessed 1 Dec 2025

Integra VIAFLO 96 Instruction Manual 125950_V21
(https://www.integra-biosciences.com/sites/default/files/documents/125950_v21_oi_viaflo_96_384_en.pdf)
Accessed 1 Dec 2025