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Version 2

Sinai SCENT TMC- Methylation Array (EPIC) V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

High throughput, proven procedure for bisulfite conversion of DNA
96-well desulphonation and recovery of bisulfite-treated DNA

High-Throughput Bisulfite Conversion of DNA

- 1 Add 5 μ l of M-Dilution Buffer to each DNA sample in a Conversion Plate and adjust the total volume to 50 μ l with water. Mix each sample by pipetting up and down.

Example: For 14 μ l of a DNA sample add 5 μ l M-Dilution Buffer and 31 μ l water.

- 2 Incubate the Conversion Plate containing the samples at 37°C for 15 minutes.

- 3 After the above incubation, add 100 μ l of the prepared CT Conversion Reagent to each sample and mix.

Note: The CT Conversion Reagent is light sensitive, so try to minimize the reaction's exposure to light whenever possible.

- 4 Incubate the Conversion Plate in the dark at 50°C for 12-16 hours (e.g., using a thermal cycler).

Note: See Appendix (page 11) for alternative incubation conditions (e.g., when using the Illumina Infinium® Methylation Assay).

- 5 Incubate the sample at 0-4°C (e.g., on ice or using a thermal cycler) for 10 minutes. Samples may be kept at 4°C for up to 20 hours.

- 6 Add 400 μ l of M-Binding Buffer to each well of a Zymo-Spin™ I-96 Binding Plate on a Collection Plate.

Note: The capacity of each well of the Binding Plate is 1.1 ml. The capacity of each well of the Collection Plate is 800 μ l. Empty the Collection Plate whenever necessary to prevent contamination of the Binding Plate contents by flow-through.

- 7 Load the samples (from Step 5) into the wells of the Zymo-Spin™ I-96 Binding Plate containing the M-Binding Buffer. Mix by pipetting up and down.

- 8 Centrifuge at $\geq 3,000 \times g$ (5,000 $\times g$ max.) for 5 minutes. Discard the flow-through.

- 9 Add 400 μ l of M-Wash Buffer to each well and centrifuge at $\geq 3,000 \times g$ for 5 minutes.

- 10 Add 200 μ l of M-Desulphonation Buffer to each well and let stand at room temperature (20-30°C) for 15-20 minutes. After this incubation, centrifuge at $\geq 3,000 \times g$ for 5



minutes.

Infinium HD Assay Methylation Protocol

- 11 Follow the Illumina Infinium HD Assay Methylation Protocol Guide. (see the reference)

Analyze the data

- 12 The data is analyzed using GenomeStudio Software (Illumina; see the reference)

Protocol references

Zymo Research EZ-96 DNA Methylation Kit (Deep-Well Format)

Infinium® HD Assay Methylation Protocol Guide

GenomeStudio: <https://www.illumina.com/products/by-type/informatics-products/microarray-software/genomestudio.html>