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Sinai SCENT TMC- Methylation Array (EPIC)

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

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

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

3 After the above incubation, add 100 µl 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 µl 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 µl. 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 ≥ 3,000 x g (5,000 x g max.) for 5 minutes. Discard the flow-through.

9 Add 400 µl of M-Wash Buffer to each well and centrifuge at ≥ 3,000 x g for 5 minutes.

10 Add 200 µl 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 ≥ 3,000 x g for 5 minutes.

Infinium HD Assay Methylation Protocol

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

Protocol references

Zymo Research EZ-96 DNA Methylation Kit (Deep-Well Format)
Infinium® HD Assay Methylation Protocol Guide