

Standard Operating Procedure - University of Bologna (Italy)

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| PROTOCOL | DNA methylation analysis |
| DATE | 28/04/2025 |
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Index

| | |
|---|---|
| 1. Purpose | 1 |
| 2. Background | 1 |
| 3. Procedure | 2 |
| 3.1. Equipment | 2 |
| 3.2. Reagents | 2 |
| 3.3. DNA extraction and DNA methylation analysis | 2 |

1. Purpose

This procedure describes the materials and the protocols used for DNA methylation analysis.

2. Background

To detect any aberrant DNA methylation (hyper- or hypomethylation) following chronic exposure to 5G (700 MHz or 3.5 GHz), samples are subjected to genome-wide methylation analysis using methylation arrays, which provide comprehensive coverage of CpG islands, genes and enhancers (930K markers can be studied).

3. Procedure

3.1. Equipment

- DNA quantification system: Victor³ 1420 Multilabel Counter (PerkinElmer Inc., Waltham, MA, USA).
- NanoPhotometer® N60 (Implen GmbH, Munich, Germany).
- Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA).
- Shaker: DMS-02500 (VWR International, Radnor, PA, USA).
- Centrifuge 5810 R (Eppendorf AG, Hamburg, Germany).
- Centrifuge Multifuge X Pro Series (Thermo Fisher Scientific, Waltham, MA, USA).
- Hybex Microsample Incubator (SciGene, Sunnyvale, CA, USA).
- Illumina Hybridization Oven (Illumina Inc., San Diego, CA, USA).
- Robot for automated array procedure: Freedom EVO (Tecan Group Ltd., Männedorf, Switzerland).
- System for array hybridization: iScan System (Illumina Inc., San Diego, CA, USA).

3.2. Reagents

- QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), cat. no. 51304.
- EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA), cat. no. D5001.
- Infinium MethylationEPIC v2.0 Kit (Illumina Inc., San Diego, CA, USA), cat. no. 20087708.
- RNase A (17,500 U) 2.5 mL (100 mg/mL; 7000 units/mL, solution) (QIAGEN, Hilden, Germany), cat. no. 19101.
- TE buffer pH 8.0 (UltraPure™): Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA), cat. no. 15575-038.
- Ethanol 96% (Carlo Erba Reagents, Cornaredo, MI, Italy).
- NaOH 2N solution (Illumina Inc., San Diego, CA, USA).

3.3. DNA extraction and DNA methylation analysis

- Cells are detached from the 35 mm cell culture dish using a cell scraper and centrifuged.
- Pellets are frozen at -80°C.
- Genomic DNA is extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNase treatment is performed during extraction.
- Genomic DNA is subjected to bisulfite conversion using the EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions.
- The Illumina array procedure is performed according to the Infinium® HD Assay Methylation Protocol Guide (#15019519 v01) without modification; the first part is performed in manual mode (until resuspension of amplified/fragmented/precipitated DNA), and the second part in automated mode (from chip hybridisation to staining).