Human Methylation 450K array

DNA was isolated from sample using OMEGA TISSUE DNA Kit according to the manufacturer's instructions. DNA (500 ng) was treated with bisulfate using an EZ DNA Methylation Gold Kit (Zymo Research, Irvine, CA), according to the manufacturer's instructions. The methylation of DNA was assayed on Illumina Infinium Human Methylation450k (HM450) Bead-Chips (Illumina, San Diego, CA) using the Illumina HD methylation assay kit from Shanghai Biotechnology Corporation.

DNA methylation data were analyzed using the methylation analysis module within BeadStudio software employing default parameters (Illumina, Inc., San Diego CA, USA). The raw intensity data were loaded to a biocondutor package "minfi". The raw data were normalized using the subset-quantile within array normalization (SWAN) method and probes with a detection p-value >= 0.01 in at least one sample were excluded from further analysis. Methylation values, referred to as β-values, were calculated as the ratio of the methylated signal intensity to the sum of the methylated and unmethylated signals after background subtraction. The β-values were reported as a DNA methylation score ranging from 0 (completely unmethylated) to 1 (fully methylated). Differentially methylated CpGs were selected using an algorithm in IMA Bioconductor. In this study, we assessed the mean-difference β-value (Δβ) between the two sample groups for each CpG site. Specifically, we considered a probe as differentially methylated if the absolute Δβ was higher than 0.14 and the statistical test was significant (p value <0.05).