Infinium HumanMethylation27 Bead Chip (Illumina)
Acute Myeloid Leukemia (AML) - Methylation

*Protocols performed at the Johns Hopkins University.

Bisulfite conversion of genomic DNA was performed with EZ DNA methylation Kit (Zymo Research, Irvine, CA, #D5002) following the manufacturer's protocol with modifications for the Infinium Methylation Assay. Briefly, one microgram of genomic DNA was mixed with 5 µl of Dilution Buffer and incubated at 37°C for 15 minutes and then mixed with 100 µl of conversion reagent prepared as instructed in the protocol. Mixtures were incubated in a thermocycler for 16 cycles at 95°C for 30 seconds and 50°C for 60 minutes. Bisulfite-converted DNA samples were loaded onto the provided 96-column plates for desulphonation, washing and elution. The concentration of bisulfite-converted, eluted DNA was measured by UV-absorbance using a NanoDrop-1000 (Thermo Fisher Scientific, Waltham, MA). Bisulfite-converted genomic DNA was analyzed using the Infinium Human Methylation27 Beadchip Kit (Illumina, San Diego, CA, #WG-311-1202). DNA amplification, fragmentation, array hybridization, extension and staining were performed with reagents provided in the kit according to the manufacturer's protocol (Illumina Infinium II Methylation Assay, #WG-901-2701). Briefly, 4 µl of bisulfite-converted genomic DNA at a minimum concentration of 20 ng/µL) was added to 0.8 ml 96-well storage plate (Thermo Fisher Scientific), denatured in 0.014N sodium hydroxide, neutralized and then amplified for 20-24 hours at 37°C. Samples were fragmented at 37°C for 60 minutes and precipitated in isopropanol. Re-suspended samples were denatured in a 96-well plate heat block at 95°C for 20 minutes. 15 µl of each sample was loaded onto a 12-sample BeadChip, assembled in the hybridization chamber as instructed by the manufacturer and incubated at 48°C for 16-20 hours. Following hybridization, the BeadChips were washed and assembled in a fluid flow-through station for primer-extension reaction and staining with reagents and buffers provided.

Polymer-coated BeadChips were scanned in an iScan scanner (Illumina) using Inf Methylation mode.

Signal intensity and Beta value data were extracted using the Methylation module of GenomeStudio (Illumina, v2011.1) software following the Methylation analysis pipeline without normalization or background subtraction using BeadChip content descriptors provided by the manufacturer (HumanMethylation27_270596_v.1.2.bpm).

Summary beta values for each locus with annotations for Illumina probe name, gene symbol, chromosome and CpG position (UCSC hg18).

Data were normalized using functional normalization (funnorm) as implemented in the minfi package and summarized as beta values [M /(M+U)] with annotation at each locus for Illumina probe name, gene symbol, chromosome and CpG position (UCSC hg19). Probes having an annotated SNV within the CpG or SBE site are masked as NA across all samples. Probes where the non-detection probability was > 0.01 are masked as NA for individual samples.