Infinium HumanMethylation450 Bead Chip (Illumina)

Osteosarcoma (OS) – Methylation

*Protocols performed at the Phoenix Children's Hospital and Baylor College of Medicine.

Bisulfite conversion of genomic DNA was performed with EZ-96 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. Bisulfite-converted genomic DNA was analyzed using the Infinium Human Methylation450K Beadchip Kit (Illumina, San Diego, CA, #WG-314-1001). 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 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 using Illumina iScan technology which outputs data in the format of IDAT files. These are then used retrieve the probe intensities and calculate the beta-values. Raw unmethylated and methylated intensities were background corrected using out-of-band correction.

Probe intensities were then color corrected using Lumi's dye bias correction algorithm. Beta-values were calculated from probe intensities and corrected for probe bias using the beta mixture quantile dilation (BMIQ) normalization method.