Infinium HumanMethylation450 Bead Chip (Illumina)

Wilms Tumor (WT) – Methylation

*Protocol performed at Ann & Robert H. Lurie Children's Hospital.

DNA was extracted from tumor samples at Nationwide Children's BioPathology Center (BPC) by using the standard BPC protocol. The DNA samples were analyzed by Pico green to verify gDNA concentration, spectrophotometry to verify DNA purity, and gel electrophoresis to verify DNA quality. DNA samples (1.5 ug) diluted in nuclease-free water were provided to the Northwestern University Genomics Core in 96-well plate format for Illumina 450K DNA methylation analysis. Randomly selected samples were tested by Northwestern University Genomics Core to verify that the correct concentration had been provided.

Nucleic acid hybridization and labeling were performed according to the manufacturer's protocol for the Illumina 450K array at the Northwestern University Genomics Core Facility. Nucleic acid labeling is completed after the hybridization step with Illumina Infinium 450K arrays.

The array scanning protocol was performed according to the manufacturer's protocol for the Illumina 450K array at the Northwestern University Genomics Core Facility.
Raw data files (1 red and 1 green .idat file per sample and 1 .sdf file from each array, which included 12 samples per array) were processed at the Northwestern University Genomics Core Facility by BeadStudio software. The following subtables were generated by BeadStudio and were downloaded in .txt format (Level 2 data): (1) the Sample Methylation Profile .txt, (2) the Control Profile .txt, and (3) the Control Probe Profile .txt. Several quality control steps were used for these data. Samples were subjected to the internal quality controls in the Bioconductor lumi package. Samples were subjected to a color balance check using the Bioconductor lumi package. Gender analysis was performed to increase our confidence that the data correctly corresponded to the expected sample. Because one of the X chromosomes is heavily methylated in females, the density of X-chromosome methylation is a good indicator of gender. Unsupervised hierarchical clustering based on all methylated regions on the X-chromosome was performed using average-linkage clustering with CLUSTER and the results were displayed with TREEVIEW. The tumors were assigned a gender based on their clustering in the tumor dendogram, which was checked against the known gender of the sample. The X-chromosome methylation profile corresponded to gender in the majority (~95%) of the samples; the other samples did not show gender-specific patterns.

The Sample Methylation Profile text, which included information for all of the samples, was broken down at the DCC into a single .txt file (level 2 data) per sample containing the following columns: (1) the sample ID, (2) probe name, (3) AVG_Beta value, (4) gene Symbol, (5) chromosome, and (6) position.