Genome-Wide Human SNP Array 6.0 (Affymetrix)

Pediatric Preclinical Testing Program (PPTP) – Copy Number

*Protocol performed at Nationwide Children's Hospital.

The DNA extraction was performed according to the Qiagen manufacturer's protocol (DNeasy Kit) in combination with Trizol.

All genotyping for the Genome-wide Human SNP array 6.0 was performed according to Affymetrix manufacturer's protocol. Briefly, two identical aliquots containing 250 ng of DNA were digested with specific restrictions enzymes in separate reactions; one reaction contained Nsp1 and the other Sty1. Immediately following digestion, each sample was ligated with adaptors containing a complementary sequence to the overhang generated at digestion. Following ligation, each sample was subjected to PCR amplification using standard reagents. Following PCR, each sample was assayed on a 2% agarose gel to ensure that a DNA smear of appropriate size was produced. The Nsp and Sty amplifications were combined, purified and quantitated. All samples with at least 180 µg total DNA were allowed to continue to fragmentation using the enzymatic reaction Affymetrix Fragmentation reagent. The fragmented DNA was assayed on a 4% agarose gel to ensure that the size of the DNA collapsed to less than 75nt. Following fragmentation, the DNA was end-labeled with terminal deoxy transferase and Affymetrix DNA labeling reagent.

Nucleic acid hybridization for the Genome-wide Human SNP array 6.0 was performed according to the manufacturer's protocol for the AffyMetrix 6.0 SNP array. Each sample was then resuspended in hybridization buffer and hybridized to the Affymetrix 6.0 array for 16 hours. Following hybridization, the arrays were washed on the Affymetrix Fluidics station and scanned on the GeneChip scanner.

The array scanning protocol for the Genome-wide Human SNP array 6.0 was performed according to the manufacturer's protocol for the AffyMetrix 6.0 SNP array.