CRL: copy number alteration - Affymetrix Genome-Wide Human SNP Array 6.0, microarray

Molecular Methods Description:

The Affymetrix Genome-Wide Human SNP Array 6.0 features 1.8 million genetic markers, including more than 906,600 single nucleotide [polymorphisms (SNPs) and more than 946,000 probes for the detection of copy number variation]. Affymetrix Genome wide SNP6.0 CEL files were loaded into the Affymetrix Genotyping Console 4.1 Software (Affymetrix). For more information on the software, please refer to the company website at this URL: Genotyping Console Software **. After scanning, arrays were checked for quality using the Affymetrix Genotyping Console. In accordance with Affymetrix guidelines, SNP6.0 arrays with a Contrast QC value above 0.4 and MAPD below 0.35 were excluded from further analysis. Copy number variations were calculated using Affymetrix GTC v4.1 and PICNIC software provided by the Cancer Genome Project from the Welcome Trust Sanger Institute (PICNIC software ** website). **DNA PREPARATION**

DNA was extracted from snap frozen tumor xenografts. Tumors were digested with proteinase K at 55°C overnight and the lysate was digested with DNAse-free RNAse (Qiagen). DNA was extracted with a mix of phenol:chloroform:isoamylalcohol and precipitated with ethanol. DNA pellets were washed and resuspended in TElow. The integrity of each DNA preparation was checked on a 1.3% agarose gel and the purity analyzed using NanoDrop 2000 (Thermo Scientific). The following QC criteria applied: - DNA purity: optical density (OD) ratios of 260/280 nm and 260/230 nm must be close to 2.0. - DNA integrity: The DNA must run in a clearly defined high molecular weight band on the agarose gel.

Only DNA which passed QC was added to the repository. The DNA concentration was adjusted to 100 ng/µl based on NanoDrop results and stored at 4°C.

Analysis Description:

PICNIC (Predicting Integral Copy Numbers In Cancer) was specifically designed to call copy numbers from Affymetrix SNP6 data. It includes normalisation of data and determination of underlying copy numbers for each segment by genome wide analysis of allele ratio and signal strength data. Additionally, the average ploidy of each sample was determined. The PICNIC segment files were further mapped to gene coordinates from the hg38 human reference genome, yielding a PICNIC copy number score for each sample and gene. For gene locations in more than 2 segments, only one final copy-number value was selected by priority order using the homozygous deletion (PICNIC score = 0) first, or the maximum PICNIC score. Copy number GAIN and LOSS were defined as suggested by COSMIC **

Table:

Copy Number Status	Average genome ploidy	PICNIC copy number score
GAIN	≤ 2.7	CN ≥ 5
	> 2.7	CN ≥ 9
AXIN2	≤ 2.7	CN = 0
	> 2.7	CN < (average genome ploidy – 2.7)