Appendix A, Supplementary Material:

DNA methylation analysis using universal beads array

A golden gate methylation Cancer pannel (Illumina) was used to quantify DNA methylation on 45 prostate cancer patients and 10 normal prostate tissues. The panel interrogate for the methylation state of 1505 CpGs sites selected from 807 cancer-related genes. Methylation assay was performed as described previously (1). Briefly, for each CpG site, four probes were designed: two allele-specific oligos (ASOs) and two locus-specific oligos (LSOs). Each ASO-LSO oligo pair corresponded to either the methylated or unmethylated state of the CpG site. Bisulfite conversion of DNA samples was done using the EZ DNA methylation kit (Zymo Research, Orange, CA). The array was hybridized under a temperature gradient program, and arrays were imaged using a BeadArray Reader (Illumina Inc.). Image processing and intensity data extraction software was used as described previously (1). Each methylation data point is represented by fluorescent signals from the M (methylated) and U (unmethylated) alleles. Background intensity computed from a set of negative controls was subtracted from each analytical data point. The beta value was then calculated as the ratio of fluorescent signals from the two alleles according to the following formula:

$$\beta\text{-value} = [\text{Max}(M,0)]/[\text{Max}(U,0) + \text{Max}(M,0) + 100]$$

The beta value is a quantitative measure of DNA methylation levels of every CpG included in the array, and ranges from 0 (completely unmethylated) to 1 (completely methylated).

1. Bibikova M, Lin ZW, Zhou LX, et al: High-throughput DNA methylation profiling using universal bead arrays. Genome Research 16:383-393, 2006