Supplementary Methods

Genomic DNA preparation and DNA methylation profiling

1. Genomic DNA quantitation

DNA samples were checked the quality using NanoDrop ND-1000 UV-Vis spectrophotometer (Wilmington, DE, USA). Then samples were electrophoresed on agarose gels and samples with intact genomic DNA showing no smearing on agarose gel electrophoresis were selected for experiment. Intact genomic DNA was diluted to 50 ng/μL concentration based on Quant-iT Picogreen (Invitrogen, Carlsbad, CA, USA) quantitation. Concentrations were adjusted based on these results. All prepared samples were bisulfite-converted according to the Zymo EZ DNA methylation kit protocols (Zymo Research, Orange, CA, USA).

2. Bisulfite conversion (Zymo EZ DNA methylation kit)

Five hundred nanograms of input gDNA was required for the bisulfite conversion. Add conversion reagent and incubate in a thermocycler to denature. CT converted DNA was washing and desulphonating with desulphonation buffer. After desulphonation, the DNA was washing again and eluting with 12 μL elution buffer.

3. Sample amplification and hybridization for BeadChips (MethylationEPIC DNA Analysis BeadChip Kit; Illumina, San Diego, CA, USA)

The whole-genome amplification process requires 200 ng of input bisulfite-converted DNA, MA1 and creates a sufficient quantity of DNA (1,000× amplification) to be used on a single BeadChip in the infinium methylation assay (Illumina RPM and MSM). After amplification, the product is fragmented using a proprietary reagent (FMS), precipitated with 2-propanol (plus precipitating reagent; PM1), and resuspended in formamide-containing hybridization buffer (RA1). The DNA samples are denatured at 95°C for 20 minutes, then placed in a humidified container for a minimum of 16 hours at 48°C allowing CpG loci to hybridize to the 50mer capture probes.

4. Allele specific single-base extension and staining on BeadChips

Following hybridization, the BeadChip/Te-Flow chamber assembly was placed on the temperature-controlled Tecan Flowthrough Chamber Rack, and all subsequent washing, extension, and staining were performed by addition of reagents to the Te-Flow chamber.

For the allele specific single-base extension assay, primers were extended with a polymerase and labeled nucleotide mix (TEM), and stained with repeated application of STM (staining reagent) and ATM (anti-staining reagent). After staining was complete, the slides were washed with low salt wash buffer (PB1), immediately coated with XC4, and then imaged on the Illumina iScan Reader.

5. Imaging the BeadChip and data analysis

The Illumina iScan Reader is a two-color (532 nm/658 nm) confocal fluorescent scanner with 0.53-μm pixel resolution. The scanner excites the fluorophors generated during signal amplification/staining of the allele-specific (one color) extension products on the BeadChips. The image intensities are extracted using Illumina's iScan Control software.

6. Raw data preparation and Statistical analysis

The quality of hybridization and overall chip performance were monitored by visual inspection of both internal quality control checks and the raw scanned data. Raw data were extracted as beta values for each CpG for each sample using R watermelon package. Beta values were calculated by subtracting background using negative controls on the array and taking the ratio of the methylated signal intensity against the sum of both methylated and unmethylated signals. A beta value of 1–1.0 was reported significant percent methylation, from 0% to 100%, respectively, for each CpG site.

Array CpG probes that have detection p-value ≥0.05 (similar to signal to noise) in over 25% samples were filtered out. (We applied a filtering criterion for data analysis; good signal value was required to obtain a detection p-value <0.05). And then filtered data was background correction & dye bias equalization by R methylumi & lumi package. To reduce Infinium I and Infinium II assay bias, corrected signal value was normalized by BMIQ (Beta Mixture Quantile) method.

Differentially expressed methylation list was determined using |delta_mean| ≥0.2 (the difference of methylation signal, avg beta of Case-avg beta of Control) and p-value <0.05 of independent t-test in which the null hypothesis was that no difference exists among two groups.

All data analysis and visualization of differentially expressed genes was conducted using R 3.0.2 (http://www.r-project.org).

References

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