# Appendix 3: Supplementary Material & Methods

## Materials

The data used in this study consists of targeted bisulfite-sequenced (84 MB target - SureSelect Methyl-Seq, Agilent Technologies, Santa Clara, CA, USA) saliva DNA from samples of 100 11 years-old Finnish females. Per individual, we measured methylation in 2,947,202 loci, mainly residing in CpG islands. The size of these islands ranged from 2 to 791 CpGs.

A custom script produced β-methylation values from raw sequencing data by combining open-source softwares. First, low quality sequences and adaptors were removed using the Nesoni clip (version 0.115) (http://www.vicbioinformatics.com/software.nesoni.shtml). The bisulfite-converted sequence reads were then mapped to the human genome (hg19) using Bowtie2 (version 2.0.5) (Langmead & Salzberg, 2012) and Bismark (version 0.10) (Krueger & Andrews, 2011). The Bismark methylation extractor and custom formatting scripts were used to calculate β-methylation values for CpG sites. The first seven bases of each sequence were ignored, as a strong bias toward non-methylation was caused by the insertion of unmethylated cytosines during end-repair in the sequencing library preparation. CpG sites at which more than 25% of the samples had less than 10x coverage were discarded.

To make the benchmarking computationally feasible, we extracted all annotated regions from chromosome 22. This chromosome had 58,910 measured CpGs, distributed over 1,071 regions, with a mean of 55 observations per region, and a range of 16 to 456 CpGs per region. Chromosome 22 represented a reduced dataset without any a priori significant regions for the phenotypes sampled in earlier study.

## Methods

Simulation parameters for Bumphunter and DMRcate

### Bumphunter

The main parameters in Bumphunter are; the maximum allowed gap between probes within a cluster, the trimming coefficient, and the number of permutations or bootstraps. The trimming coefficient gives the quantile of the test statistics that are aggregated into new clusters in each permutation. This was set to the default value of 0.99, thus setting the threshold to include 1% of the top hits. We only considered regions with a “fwer” value below 0.05, by setting the null method to permutations, and B to 2000. We did not smooth the beta-values since the auto-correlation did indicate sufficient smoothness.

### DMRcate

The genome wide significance level for the probe-wise p-values is set by the user as well as the multiple testing adjustment method for the probe-wise p-value. The scaling factor (C) is inverse proportional to the standard deviation of the kernel smoothing. Empirical testing by the authors of DMRcate showed that when smoothing parameter is 1kb, the optimal scaling factor was close to 50 for sequencing data (Peters et al., 2015).

## References:

Krueger, F. and S. R. Andrews (2011). "Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications." Bioinformatics **27**(11): 1571-1572.

Langmead, B. and S. L. Salzberg (2012). "Fast gapped-read alignment with Bowtie 2." Nature methods **9**(4): 357-359.

Peters, T. J., M. J. Buckley, A. L. Statham, R. Pidsley, K. Samaras, et al. (2015). "De novo identification of differentially methylated regions in the human genome." Epigenetics Chromatin **8**: 6.