

The **DMRcate** package user's guide

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Summary

DMRcate extracts the most differentially methylated regions (DMRs) and variably methylated regions (VMRs) from Illumina®Infinium HumanMethylation450 BeadChip (hereinafter referred to as the 450k array) samples via kernel smoothing. We provide clean, transparent code and highly interpretable and exportable results.

```
source("http://bioconductor.org/biocLite.R")
biocLite("DMRcate")
```

Load **DMRcate** into the workspace:

```
library(DMRcate)
```

We now can load in the test data set of beta values. We assume at this point that normalisation and filtering out bad-quality probes via their detection p -values have already been done. Many packages are available for these purposes, including **minfi**, **watermelon** and **methylumi**. M-values (logit-transform of beta) are preferable to beta values for significance testing via **limma** because of increased sensitivity, but we will retain the beta matrix for visualisation purposes later on.

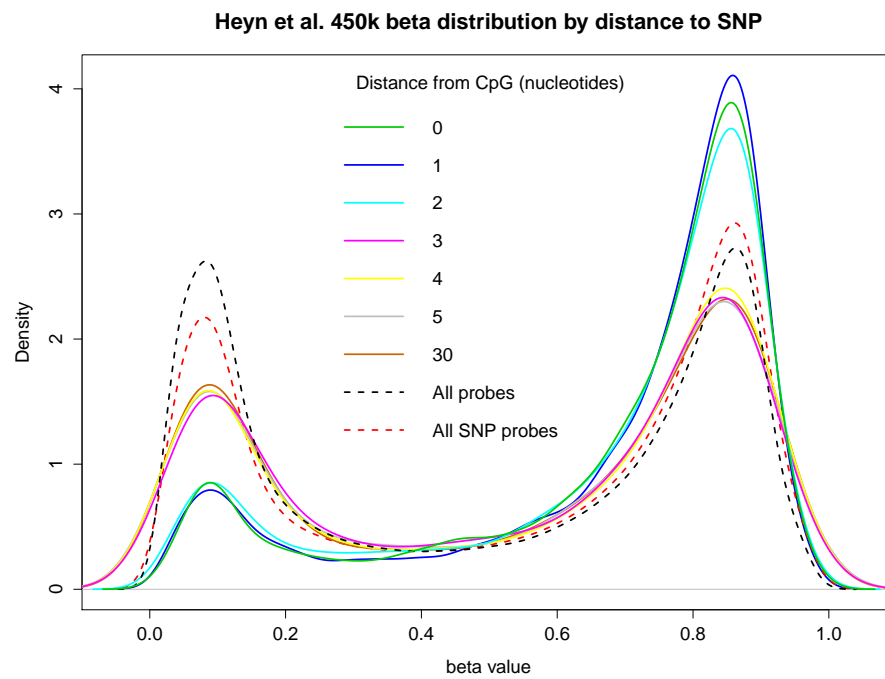
The TCGA (Cancer Genome Atlas - colorectal cancer) data in **myBetas** only comes from chromosome 20, but **DMRcate** will have no problem taking in the approximately half million probes as input for this pipeline either.

```
data(dmrctedata)
myMs <- logit2(myBetas)
```

Some of the methylation measurements on the array may be confounded by proximity to SNPs, and cross-hybridisation to other areas of the genome[1]. In particular, probes that are 0, 1, or 2 nucleotides from the methylcytosine of interest show a markedly different distribution to those farther away, in healthy tissue (Figure 1).

It is with this in mind that we filter out probes 2 nucleotides or closer to a SNP that have a minor allele frequency greater than 0.05, and the approximately 30,000 [1] cross-reactive probes, so as to reduce confounding. Here we

Figure 1: Beta distribution of 450K probes from publically available data from blood samples of healthy individuals [2] by their proximity to a SNP. “All SNP probes” refers to the 153 113 probes listed by Illumina® whose values may potentially be confounded by a SNP.



use Illumina®'s database of approximately 150,000 potentially SNP-confounded probes, and an internally-loaded dataset of the probes from [1], to filter these probes out. About 600 are removed from our M-matrix of approximately 10,000:

```
nrow(illuminaSNPs)

## [1] 153113

nrow(myMs)

## [1] 10042

myMs.noSNPs <- rmSNPandCH(myMs, dist=2, mafcut=0.05)
nrow(myMs.noSNPs)

## [1] 9403
```

Next we want to annotate our matrix of M-values with relevant information. The default is the `ilmn12.hg19` annotation, but this can be substituted for any argument compatible with the interface provided by the `minfi` package. We also use the backbone of the `limma` pipeline for differential array analysis to get *t*-statistics changes and, optionally, filter probes by their *fdr*-corrected *p*-value. Here we have 38 patients with 2 tissue samples each taken from them. We want to compare within patients across tissue samples, so we set up our variables for a standard `limma` pipeline, and set `coef=39` in `cpg.annotate` since this corresponds to the phenotype comparison in `design`.

```
patient <- factor(sub("-", "*", "", colnames(myMs)))
type <- factor(sub(".*-", "", colnames(myMs)))
design <- model.matrix(~patient + type)
myannotation <- cpg.annotate(myMs.noSNPs, analysis.type="differential",
                             design=design, coef=39)

## Your contrast returned 6101 individually significant probes. We
## recommend the default setting of pcutoff in dmrcate().
## Loading required package: IlluminaHumanMethylation450kanno.ilmn12.hg19
```

Now we can find our most differentially methylated regions with `dmrcate`.

For each chromosome, two smoothed estimates are computed: one weighted with `myannotation$weights` and one not, for a null comparison. The two estimates are compared via a Satterthwaite approximation[3], and a significance test is calculated at all hg19 coordinates that an input probe maps to. After *fdr*-correction, regions are then agglomerated from groups of significant probes where the distance to the next consecutive probe is less than `lambda` nucleotides.

```
dmrcoutput <- dmrcate(myannotation, lambda=1000, C=2)
```

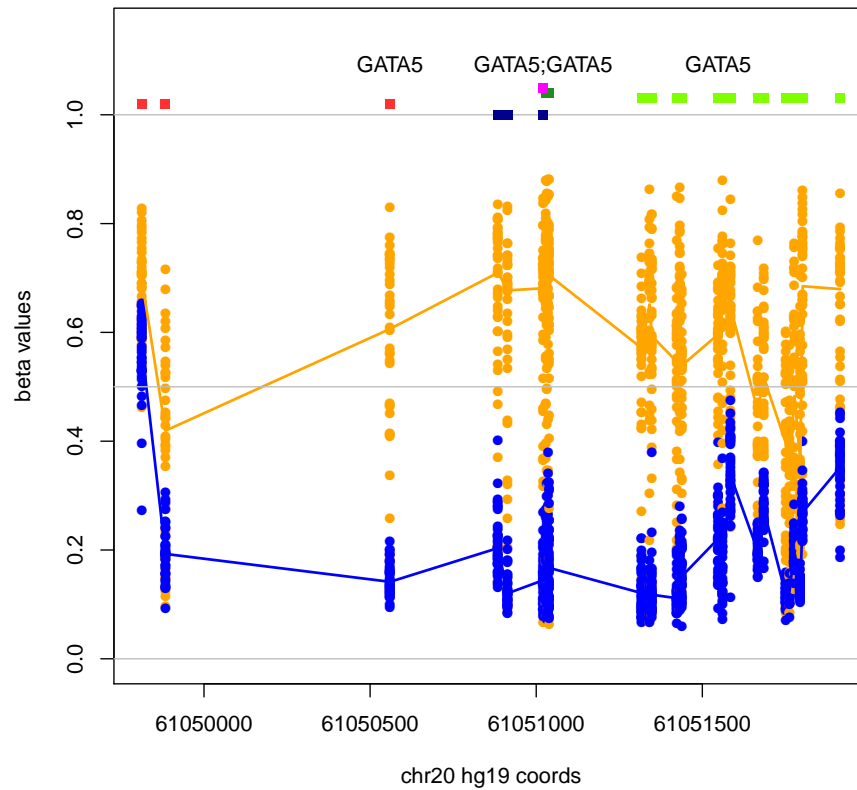
```
## Fitting chr20...
## Demarcating regions...
## Done!
```

Now we can plot a significant DMR. We'll choose one associated with the GATA5 locus.

```
head(dmrcoutput$results)
```

```
##      gene_assoc                                     group
## 839 GNASAS,GNAS Body,3'UTR,TSS200,TSS1500,5'UTR,1stExon
## 934      GATA5      Body,5'UTR,1stExon,TSS200,TSS1500
## 993  MIR124-3      TSS1500,TSS200,Body
## 554      TOX2      TSS1500,TSS200,Body,5'UTR,1stExon
## 275  TMEM90B      TSS1500,TSS200,5'UTR,1stExon
## 283      VSX1      Body,1stExon,5'UTR,TSS200,TSS1500
##
##      hg19coord no.probes minpval      meanpval      maxbetafc
## 839 chr20:57424521-57431303      77      0 3.002698e-29 -0.2084268
## 934 chr20:61049813-61051915      27      0 2.924163e-74 0.4770680
## 993 chr20:61806628-61810795      23      0 5.818155e-24 0.4182034
## 554 chr20:42543034-42545099      22      0 1.177014e-33 0.3684618
## 275 chr20:24448859-24452131      21      0 3.740710e-113 0.4263522
## 283 chr20:25061762-25065553      20      0 1.070921e-45 0.4679376
```

```
DMR.plot(dmrcoutput=dmrcoutput, dmr=2, betas=myBetas,
         phen.col=c(rep("orange", 38), rep("blue", 38)),
         pch=16, toscale=TRUE, plotmedians=TRUE)
```



```
sessionInfo()

## R version 3.2.1 (2015-06-18)
## Platform: x86_64-apple-darwin10.8.0 (64-bit)
## Running under: OS X 10.6.8 (Snow Leopard)
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4      parallel    stats      graphics  grDevices  utils      datasets
## [8] methods     base
##
## other attached packages:
## [1] IlluminaHumanMethylation450kanno.ilmn12.hg19_0.2.1
## [2] DMRcate_1.4.2
```

```

## [3] DMRcatedata_1.4.0
## [4] minfi_1.14.0
## [5] bumphunter_1.8.0
## [6] locfit_1.5-9.1
## [7] iterators_1.0.7
## [8] foreach_1.4.2
## [9] Biostrings_2.36.1
## [10] XVector_0.8.0
## [11] GenomicRanges_1.20.5
## [12] GenomeInfoDb_1.4.1
## [13] IRanges_2.2.5
## [14] S4Vectors_0.6.1
## [15] lattice_0.20-31
## [16] Biobase_2.28.0
## [17] BiocGenerics_0.14.0
## [18] limma_3.24.12
##
## loaded via a namespace (and not attached):
## [1] genefilter_1.50.0      splines_3.2.1
## [3] beanplot_1.2          rtracklayer_1.28.5
## [5] GenomicFeatures_1.20.1 XML_3.98-1.2
## [7] survival_2.38-2       DBI_0.3.1
## [9] BiocParallel_1.2.6    RColorBrewer_1.1-2
## [11] registry_0.2          rngtools_1.2.4
## [13] lambda.r_1.1.7        doRNG_1.6
## [15] matrixStats_0.14.2    plyr_1.8.3
## [17] pkgmaker_0.22         stringr_1.0.0
## [19] zlibbioc_1.14.0       futile.logger_1.4.1
## [21] codetools_0.2-11      evaluate_0.7
## [23] knitr_1.10.5          biomaRt_2.24.0
## [25] AnnotationDbi_1.30.1  illuminaio_0.10.0
## [27] preprocessCore_1.30.0 highr_0.5
## [29] Rcpp_0.11.6          xtable_1.7-4
## [31] formatR_1.2           base64_1.1
## [33] annotate_1.46.0        Rsamtools_1.20.4
## [35] digest_0.6.8          stringi_0.5-5
## [37] nor1mix_1.2-0         grid_3.2.1
## [39] GEOquery_2.34.0       quadprog_1.5-5
## [41] tools_3.2.1          bitops_1.0-6
## [43] magrittr_1.5          siggenes_1.42.0
## [45] RCurl_1.95-4.6        RSQLite_1.0.0
## [47] futile.options_1.0.0  MASS_7.3-41
## [49] reshape_0.8.5         mclust_5.0.1
## [51] nlme_3.1-120          GenomicAlignments_1.4.1
## [53] multtest_2.24.0

```

References

- [1] Chen YA, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, Zanke BW, Gallinger S, Hudson TJ, Weksberg R. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics*. 2013 Jan 11;8(2).
- [2] Heyn H, Li N, Ferreira HJ, Moran S, Pisano DG, Gomez A, Esteller M. Distinct DNA methylomes of newborns and centenarians. *Proceedings of the National Academy of Sciences*. 2012 **109**(26), 10522-7.
- [3] Satterthwaite, F. E. (1946), An Approximate Distribution of Estimates of Variance Components., *Biometrics Bulletin*. 1946 **2**: 110-114