Calling DMRs from EPICv1 and 450K data

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Summary

This vignette demonstrates how to call DMRs from older versions of Illumina arrays, namely 450K and EPICv1 (pre-2022).

```
if (!require("BiocManager"))
        install.packages("BiocManager")
BiocManager::install("DMRcate")
```

Load `DMRcate` into the workspace:

```
library(DMRcate)
```

For this vignette, we will demonstrate DMRcate's array utility using data from `ExperimentHub`, namely Illumina HumanMethylationEPIC data from the data packages `FlowSorted.Blood.EPIC`. Specifically, we are interested in the methylation differences between CD4+ and CD8+ T cells.

```
library(ExperimentHub)
eh <- ExperimentHub()
FlowSorted.Blood.EPIC <- eh[["EH1136"]]
tcell <- FlowSorted.Blood.EPIC[,colData(FlowSorted.Blood.EPIC)$CD4T==100 |
                                colData(FlowSorted.Blood.EPIC)$CD8T==100]
```

¡¡chr2, Firstly we filter out any probes where any sample has a failed position. Then we normalise using `minfi::preprocessFunnorm`. For this vignette, we will restrict the analysis to chromosome 2. After this, we extract the M-values from the GenomicRatioSet.

```
detP <- detectionP(tcell)

## Loading required package:  IlluminaHumanMethylationEPICmanifest

remove <- apply(detP, 1, function (x) any(x > 0.01))
tcell <- preprocessFunnorm(tcell)
```

```
## [preprocessFunnorm] Background and dye bias correction with noob
## Loading required package:  IlluminaHumanMethylationEPICanno.ilm10b4.hg19
## [preprocessFunnorm] Mapping to genome
## [preprocessFunnorm] Quantile extraction
## [preprocessFunnorm] Normalization

tcell <- tcell[seqnames(tcell) %in% "chr2",]
tcell <- tcell[!rownames(tcell) %in% names(which(remove)),]
tcellms <- getM(tcell)
```

M-values (logit-transform of beta) are preferable to beta values for significance testing via `limma` since they approximate normality, and provide greater sensitivity towards the extremes of the distribution, but we will use a beta matrix for visualisation purposes later on.

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, 2]. 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 48,000 [1, 2] cross-reactive probes on either 450K and/or EPIC, so as to reduce confounding. Here we use a combination of *in silico* analyses from [1, 2]. About 4,000 are removed from our M-matrix of 64,729 chromosome 2 probes:

```
nrow(tcellms)

## [1] 64729

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

## [1] 60445
```

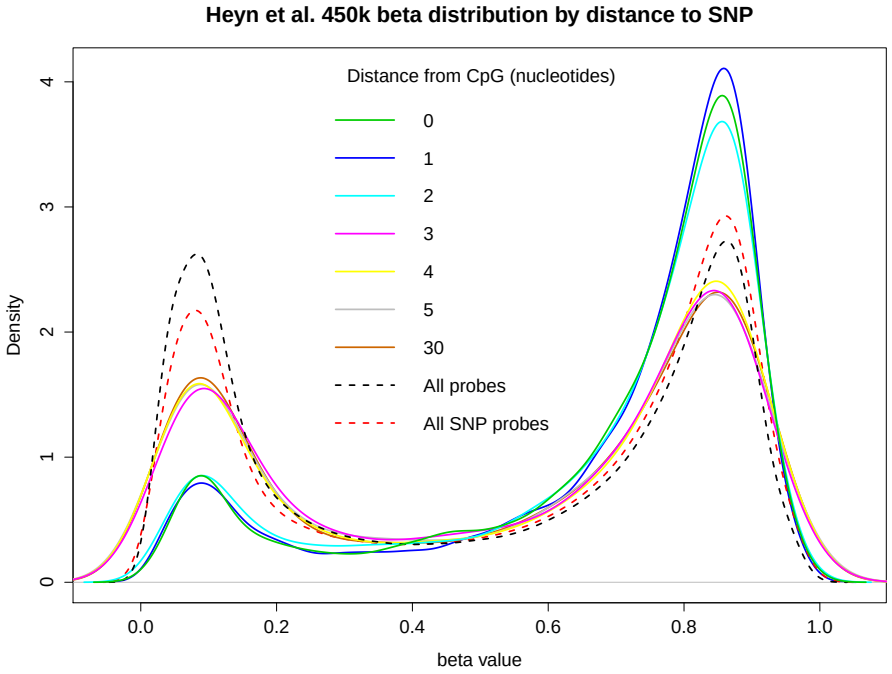
Here we have 6 CD8+ T cell assays, and 7 CD4+ T cell assays; we want to call DMRs between these groups. One of the CD4+ assays is a technical replicate, so we will average these two replicates like so:

```
tcell$Replicate

##  [1] ""          ""          ""          ""          ""          ""
##  [7] ""          ""          ""          "Th2535-1" "Th2535-1" ""
## [13] ""

tcell$Replicate[tcell$Replicate==""] <- tcell$Sample_Name[tcell$Replicate==""]
tcellms.noSNPs <- limma::avearrays(tcellms.noSNPs, tcell$Replicate)
tcell <- tcell[,!duplicated(tcell$Replicate)]
```

Figure 1: Beta distribution of 450K probes from publicly available data from blood samples of healthy individuals [3] 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.



```
tcell <- tcell[rownames(tcellms.noSNPs),]
colnames(tcellms.noSNPs) <- colnames(tcell)
assays(tcell)[["M"]] <- tcellms.noSNPs
assays(tcell)[["Beta"]] <- ilogit2(tcellms.noSNPs)
```

Next we want to annotate our matrix of M-values with relevant information. We also use the backbone of the `limma` pipeline for differential array analysis. We want to compare within patients across tissue samples, so we set up our variables for a standard limma pipeline, and set `coef=2` in `cpg.annotate()` since this corresponds to the phenotype comparison in `design`.

`cpg.annotate()` takes either a data matrix with Illumina probe IDs, or an already prepared GenomicRatioSet from `minfi`.

```
type <- factor(tcell$CellType)
design <- model.matrix(~type)
myannotation <- cpg.annotate("array", tcell, arraytype = "EPICv1",
                             analysis.type="differential", design=design, coef=2)
```

```
myannotation

## CpGannotated object describing 60445 CpG sites, with independent
## CpG threshold indexed at fdr=0.05 and 2710 significant CpG sites.
```

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

For each chromosome, two smoothed estimates are computed: one weighted with per-CpG t-statistics and one not, for a null comparison. The two estimates are compared via a Satterthwaite approximation[4], and a significance test is calculated at all hg19 coordinates that an input probe maps to. After fdr-correction, regions are then aggregated from groups of post-smoothed significant probes where the distance to the next consecutive probe is less than `lambda` nucleotides.

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

## Fitting chr2...
## Demarcating regions...
## Done!

dmrcoutput

## DMResults object with 439 DMRs.
## Use extractRanges() to produce a GRanges object of these.
```

We can convert our DMR list to a GRanges object, which uses the `genome` argument to annotate overlapping gene loci.

```
results.ranges <- extractRanges(dmrcoutput, genome = "hg19")
results.ranges
```

```
## GRanges object with 439 ranges and 8 metadata columns:
##         seqnames              ranges strand |   no.cpgs min_smoothed_fdr
##            <Rle>           <IRanges>  <Rle> | <integer>        <numeric>
##     [1]     chr2   87014979-87021117      * |        26      0.00000e+00
##     [2]     chr2 234294036-234297039      * |        14     1.31193e-103
##     [3]     chr2   86991846-86992657      * |         3     2.82449e-203
##     [4]     chr2 112939119-112941244      * |         6      3.49350e-93
##     [5]     chr2 197124443-197125372      * |         4     1.36472e-135
##     ...      ...                 ...    ... .       ...              ...
##   [435]     chr2   43454761-43455773      * |        15      2.70362e-14
##   [436]     chr2 177001256-177001263      * |         3      6.91077e-10
##   [437]     chr2 121200209-121200256      * |         2      7.67192e-10
##   [438]     chr2 177052527-177053018      * |         5      2.38880e-10
##   [439]     chr2 173940027-173940121      * |         2      7.61333e-10
##           Stouffer       HMFDR      Fisher    maxdiff   meandiff
##          <numeric>   <numeric>   <numeric>  <numeric>  <numeric>
##     [1] 1.72294e-54 3.18747e-07 5.43315e-68  -0.733427  -0.236312
##     [2] 2.28737e-17 8.93582e-06 1.78156e-19  -0.385476  -0.139055
##     [3] 6.13508e-18 2.11325e-07 5.99765e-17  -0.530621  -0.395736
##     [4] 5.62342e-17 2.22940e-06 8.23449e-17   0.476714   0.336035
##     [5] 8.10953e-17 1.43019e-06 6.91821e-16   0.380658   0.287335
##     ...         ...         ...         ...        ...        ...
##   [435]    0.489338   0.0335443    0.205729 -0.0748315 -0.0133684
##   [436]    0.130045   0.2290845    0.211291  0.0456072  0.0272108
##   [437]    0.217200   0.1787023    0.226449  0.0408411  0.0400848
##   [438]    0.463773   0.1395391    0.250201  0.0408253  0.0215292
##   [439]    0.357791   0.1553425    0.253507  0.0526885  0.0306643
##         overlapping.genes
##               <character>
##     [1]              CD8A
##     [2]  DGKD, AC019221.4
##     [3]            RMND5A
##     [4]             FBLN7
##     [5] AC020571.3, HECW2
##     ...               ...
##   [435]            THADA
##   [436]          HOXD-AS2
##   [437]              <NA>
##   [438]          HOXD-AS1
##   [439]              <NA>
##   -------
##   seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

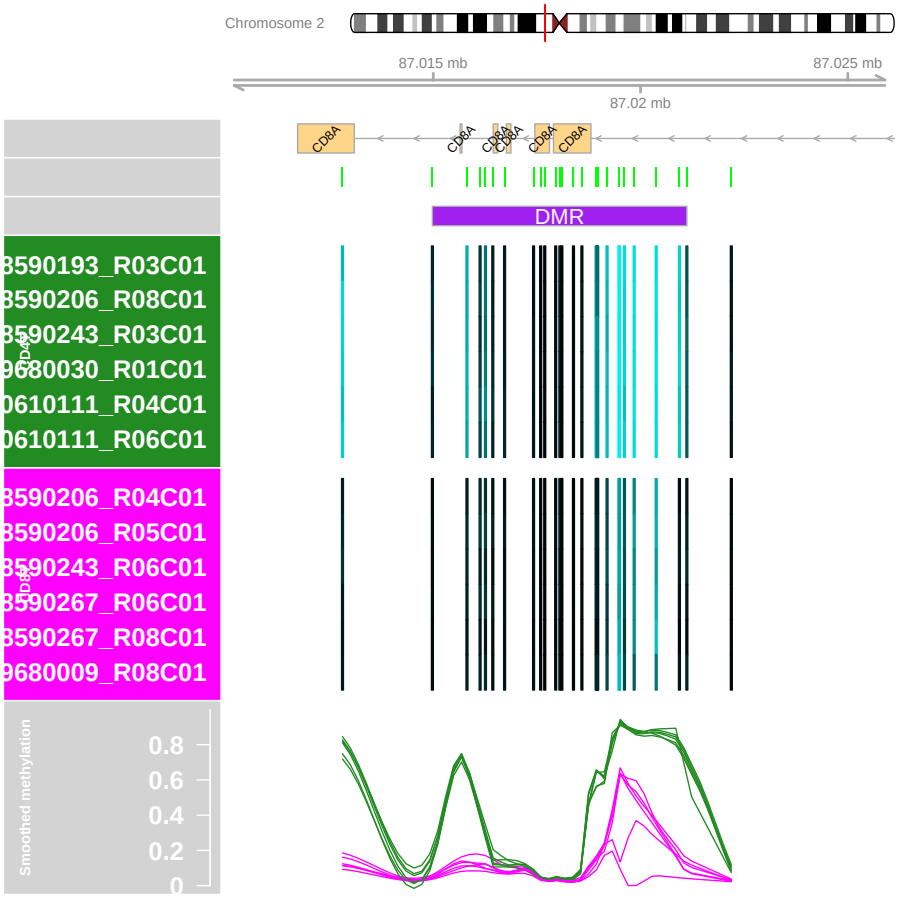
DMRs are ranked by Fisher's multiple comparison statistic, but `Stouffer` scores and the harmonic mean of the individual component FDRs (`HMFDR`) are also given in this object as alternative options for ranking DMR significance.

We can then pass this GRanges object to `DMR.plot()`, which uses the `Gviz` package as a backend for contextualising each DMR.

```
groups <- c(CD8T="magenta", CD4T="forestgreen")
cols <- groups[as.character(type)]
cols
```

```
##          CD4T          CD8T          CD8T          CD4T          CD4T
## "forestgreen"     "magenta"     "magenta" "forestgreen" "forestgreen"
##          CD8T          CD8T          CD8T          CD8T          CD4T
##     "magenta"     "magenta"     "magenta"     "magenta" "forestgreen"
##          CD4T          CD4T
## "forestgreen" "forestgreen"
```

```
DMR.plot(ranges=results.ranges, dmr=1, CpGs=getBeta(tcell), what="Beta",
         arraytype = "EPICv1", phen.col=cols, genome="hg19")
```



Consonant with the expected biology, our top DMR shows the CD8+ T cells hypomethylated across parts of the CD8A locus. The two distinct hypomethylated sections have been merged because they are less than 1000 bp apart - specified by `lambda` in the call to `dmrcate()`. To call these as separate DMRs, make `lambda` smaller.

```
sessionInfo()

## R version 4.4.0 (2024-04-24)
## Platform: x86_64-pc-linux-gnu
## Running under: Ubuntu 22.04.4 LTS
##
## Matrix products: default
## BLAS:   /home/biocbuild/bbs-3.19-bioc/R/lib/libRblas.so
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.10.0
##
```

```
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_GB              LC_COLLATE=C
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## time zone: America/New_York
## tzcode source: system (glibc)
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] DMRcatedata_2.22.0
##  [2] IlluminaHumanMethylationEPICanno.ilm10b4.hg19_0.6.0
##  [3] IlluminaHumanMethylationEPICmanifest_0.3.0
##  [4] FlowSorted.Blood.EPIC_2.8.0
##  [5] minfi_1.50.0
##  [6] bumphunter_1.46.0
##  [7] locfit_1.5-9.9
##  [8] iterators_1.0.14
##  [9] foreach_1.5.2
## [10] Biostrings_2.72.1
## [11] XVector_0.44.0
## [12] SummarizedExperiment_1.34.0
## [13] Biobase_2.64.0
## [14] MatrixGenerics_1.16.0
## [15] matrixStats_1.3.0
## [16] GenomicRanges_1.56.1
## [17] GenomeInfoDb_1.40.1
## [18] IRanges_2.38.0
## [19] S4Vectors_0.42.0
## [20] ExperimentHub_2.12.0
## [21] AnnotationHub_3.12.0
## [22] BiocFileCache_2.12.0
## [23] dbplyr_2.5.0
## [24] BiocGenerics_0.50.0
## [25] DMRcate_3.0.2
##
## loaded via a namespace (and not attached):
##   [1] splines_4.4.0
##   [2] BiocIO_1.14.0
```

```
##   [3] bitops_1.0-7
##   [4] filelock_1.0.3
##   [5] cellranger_1.1.0
##   [6] tibble_3.2.1
##   [7] R.oo_1.26.0
##   [8] preprocessCore_1.66.0
##   [9] XML_3.99-0.16.1
##  [10] rpart_4.1.23
##  [11] lifecycle_1.0.4
##  [12] httr2_1.0.1
##  [13] edgeR_4.2.0
##  [14] base64_2.0.1
##  [15] MASS_7.3-61
##  [16] lattice_0.22-6
##  [17] ensembldb_2.28.0
##  [18] scrime_1.3.5
##  [19] backports_1.5.0
##  [20] magrittr_2.0.3
##  [21] limma_3.60.3
##  [22] Hmisc_5.1-3
##  [23] rmarkdown_2.27
##  [24] yaml_2.3.8
##  [25] doRNG_1.8.6
##  [26] askpass_1.2.0
##  [27] Gviz_1.48.0
##  [28] DBI_1.2.3
##  [29] RColorBrewer_1.1-3
##  [30] abind_1.4-5
##  [31] zlibbioc_1.50.0
##  [32] quadprog_1.5-8
##  [33] purrr_1.0.2
##  [34] R.utils_2.12.3
##  [35] AnnotationFilter_1.28.0
##  [36] biovizBase_1.52.0
##  [37] RCurl_1.98-1.14
##  [38] nnet_7.3-19
##  [39] VariantAnnotation_1.50.0
##  [40] rappdirs_0.3.3
##  [41] GenomeInfoDbData_1.2.12
##  [42] genefilter_1.86.0
##  [43] annotate_1.82.0
##  [44] permute_0.9-7
##  [45] DelayedMatrixStats_1.26.0
##  [46] codetools_0.2-20
##  [47] DelayedArray_0.30.1
```

```
##  [48] xml2_1.3.6
##  [49] tidyselect_1.2.1
##  [50] UCSC.utils_1.0.0
##  [51] beanplot_1.3.1
##  [52] base64enc_0.1-3
##  [53] illuminaio_0.46.0
##  [54] GenomicAlignments_1.40.0
##  [55] jsonlite_1.8.8
##  [56] multtest_2.60.0
##  [57] Formula_1.2-5
##  [58] survival_3.7-0
##  [59] missMethyl_1.38.0
##  [60] tools_4.4.0
##  [61] progress_1.2.3
##  [62] Rcpp_1.0.12
##  [63] glue_1.7.0
##  [64] gridExtra_2.3
##  [65] SparseArray_1.4.8
##  [66] xfun_0.44
##  [67] dplyr_1.1.4
##  [68] HDF5Array_1.32.0
##  [69] withr_3.0.0
##  [70] IlluminaHumanMethylation450kanno.ilmn12.hg19_0.6.1
##  [71] BiocManager_1.30.23
##  [72] fastmap_1.2.0
##  [73] latticeExtra_0.6-30
##  [74] rhdf5filters_1.16.0
##  [75] fansi_1.0.6
##  [76] openssl_2.2.0
##  [77] digest_0.6.35
##  [78] mime_0.12
##  [79] R6_2.5.1
##  [80] colorspace_2.1-0
##  [81] gtools_3.9.5
##  [82] jpeg_0.1-10
##  [83] dichromat_2.0-0.1
##  [84] biomaRt_2.60.0
##  [85] RSQLite_2.3.7
##  [86] R.methodsS3_1.8.2
##  [87] tidyr_1.3.1
##  [88] utf8_1.2.4
##  [89] generics_0.1.3
##  [90] data.table_1.15.4
##  [91] rtracklayer_1.64.0
##  [92] prettyunits_1.2.0
```

```
##  [93] httr_1.4.7
##  [94] htmlwidgets_1.6.4
##  [95] S4Arrays_1.4.1
##  [96] pkgconfig_2.0.3
##  [97] gtable_0.3.5
##  [98] blob_1.2.4
##  [99] siggenes_1.78.0
## [100] htmltools_0.5.8.1
## [101] ProtGenerics_1.36.0
## [102] scales_1.3.0
## [103] png_0.1-8
## [104] knitr_1.47
## [105] rstudioapi_0.16.0
## [106] tzdb_0.4.0
## [107] rjson_0.2.21
## [108] nlme_3.1-165
## [109] checkmate_2.3.1
## [110] curl_5.2.1
## [111] org.Hs.eg.db_3.19.1
## [112] cachem_1.1.0
## [113] rhdf5_2.48.0
## [114] stringr_1.5.1
## [115] BiocVersion_3.19.1
## [116] foreign_0.8-86
## [117] AnnotationDbi_1.66.0
## [118] restfulr_0.0.15
## [119] GEOquery_2.72.0
## [120] pillar_1.9.0
## [121] grid_4.4.0
## [122] reshape_0.8.9
## [123] vctrs_0.6.5
## [124] xtable_1.8-4
## [125] cluster_2.1.6
## [126] htmlTable_2.4.2
## [127] evaluate_0.24.0
## [128] bsseq_1.40.0
## [129] readr_2.1.5
## [130] GenomicFeatures_1.56.0
## [131] cli_3.6.2
## [132] compiler_4.4.0
## [133] Rsamtools_2.20.0
## [134] rngtools_1.5.2
## [135] rlang_1.1.4
## [136] crayon_1.5.2
## [137] nor1mix_1.3-3
```

```
## [138] mclust_6.1.1
## [139] interp_1.1-6
## [140] plyr_1.8.9
## [141] stringi_1.8.4
## [142] deldir_2.0-4
## [143] BiocParallel_1.38.0
## [144] munsell_0.5.1
## [145] lazyeval_0.2.2
## [146] Matrix_1.7-0
## [147] BSgenome_1.72.0
## [148] hms_1.1.3
## [149] sparseMatrixStats_1.16.0
## [150] bit64_4.0.5
## [151] ggplot2_3.5.1
## [152] Rhdf5lib_1.26.0
## [153] KEGGREST_1.44.0
## [154] statmod_1.5.0
## [155] highr_0.11
## [156] memoise_2.0.1
## [157] bit_4.0.5
## [158] readxl_1.4.3
```

References

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[3] 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.

[4] Satterthwaite FE. An Approximate Distribution of Estimates of Variance Components., *Biometrics Bulletin*. 1946 **2**: 110-114