

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]
```

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
```

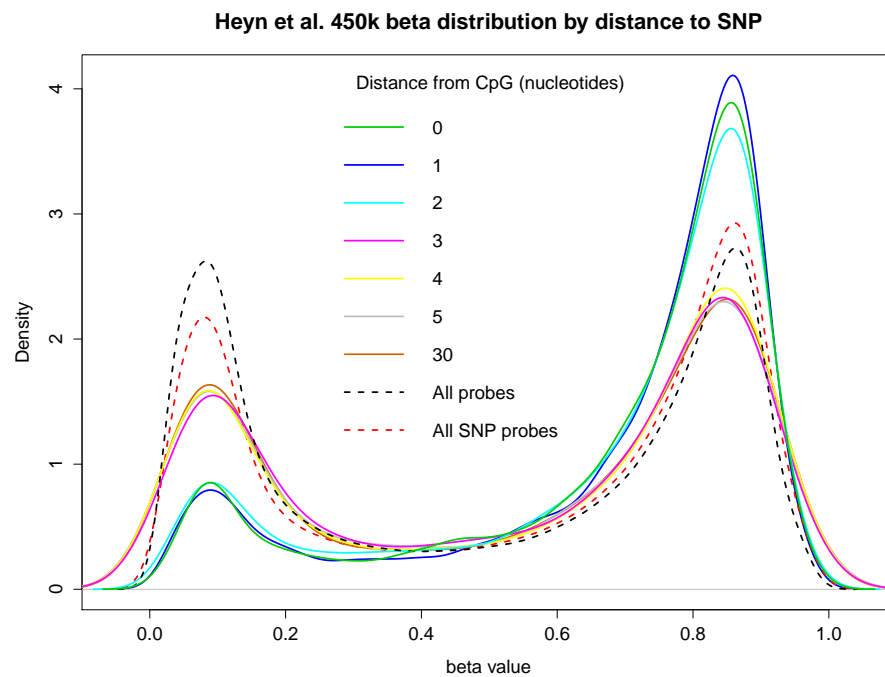
Here we have 6 CD8+ T cell assays, and 7 CD4+ T cell assays; we want to call DMRs between these groups. One of the CD4+ assays is a technical replicate, so we will average these two replicates like so:

```
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

## DMRResults 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      55757156-55758066      * |           3      1.60075e-217
##      [3]      chr2      86991846-86992657      * |           3      2.82449e-203
##      [4]      chr2      47382287-47383720      * |          14      1.25063e-192
##      [5]      chr2      16804409-16805111      * |           5      2.04178e-186
##      ...      ...      ...      ...      ...      ...
##     [435]      chr2 177001256-177001263      * |           3      6.91077e-10
##     [436]      chr2 173940027-173940121      * |           2      7.61333e-10
##     [437]      chr2 121200209-121200256      * |           2      7.67192e-10
##     [438]      chr2   97653250-97653274      * |           2      8.57482e-10
##     [439]      chr2 235372794-235372807      * |           2      9.29532e-10
##           Stouffer           HMFDR           Fisher      maxdiff      meandiff
##           <numeric>      <numeric>      <numeric> <numeric> <numeric>
##      [1] 7.16441e-104 2.37743e-09 7.62396e-121 -0.733427 -0.2363118
##      [2] 2.41969e-12 2.11366e-09 2.95110e-18  0.541286  0.3581240
##      [3] 6.90089e-26 2.73817e-09 1.08991e-24 -0.530621 -0.3957358
##      [4] 2.75767e-14 1.11231e-08 7.36384e-23 -0.390790 -0.0725018
##      [5] 4.72125e-13 7.23394e-09 4.86124e-18  0.379510  0.1485379
##      ...      ...      ...      ...      ...
##     [435] 4.81046e-04 0.021578272 1.35216e-03 0.0456072 0.0272108
##     [436] 1.90391e-02 0.009898381 1.30850e-02 0.0526885 0.0306643
##     [437] 5.20056e-03 0.013052579 6.73553e-03 0.0408411 0.0400848
##     [438] 1.88832e-03 0.019643093 3.49786e-03 0.0376177 0.0317424
##     [439] 6.15414e-06 0.000740696 1.17971e-05 0.0849654 0.0779999
##           overlapping.genes
##           <character>
##      [1]      CD8A
##      [2]      CCDC104
##      [3]      RMND5A
##      [4] C2orf61, RP11-761B3.1
##      [5]      FAM49A
##      ...      ...
##     [435]      HOXD-AS2
##     [436]      <NA>
##     [437]      <NA>
##     [438]      FAM178B
##     [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=myannotation, 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 4.6.0 (2026-04-24)
## Platform: x86_64-apple-darwin20
## Running under: macOS Monterey 12.7.6
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/4.6-x86_64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.6-x86_64/Resources/lib/libRlapack.dylib
##
```

```

## locale:
## [1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: America/New_York
## tzcode source: internal
##
## attached base packages:
## [1] parallel stats4 stats graphics grDevices utils datasets
## [8] methods base
##
## other attached packages:
## [1] DMRcatedata_2.30.0
## [2] IlluminaHumanMethylationEPICanno.ilm10b4.hg19_0.6.0
## [3] IlluminaHumanMethylationEPICmanifest_0.3.0
## [4] FlowSorted.Blood.EPIC_2.16.0
## [5] minfi_1.58.0
## [6] bumphunter_1.54.0
## [7] locfit_1.5-9.12
## [8] iterators_1.0.14
## [9] foreach_1.5.2
## [10] Biostrings_2.80.1
## [11] XVector_0.52.0
## [12] SummarizedExperiment_1.42.0
## [13] Biobase_2.72.0
## [14] MatrixGenerics_1.24.0
## [15] matrixStats_1.5.0
## [16] GenomicRanges_1.64.0
## [17] Seqinfo_1.2.0
## [18] IRanges_2.46.0
## [19] S4Vectors_0.50.1
## [20] ExperimentHub_3.2.0
## [21] AnnotationHub_4.2.0
## [22] BiocFileCache_3.2.0
## [23] dbplyr_2.5.2
## [24] BiocGenerics_0.58.1
## [25] generics_0.1.4
## [26] DMRcate_3.8.0
##
## loaded via a namespace (and not attached):
## [1] splines_4.6.0
## [2] BiocIO_1.22.0
## [3] bitops_1.0-9
## [4] filelock_1.0.3
## [5] cellranger_1.1.0
## [6] tibble_3.3.1

```



```
## [7] R.oo_1.27.1
## [8] preprocessCore_1.74.0
## [9] XML_3.99-0.23
## [10] rpart_4.1.27
## [11] lifecycle_1.0.5
## [12] httr2_1.2.2
## [13] edgeR_4.10.1
## [14] base64_2.0.2
## [15] lattice_0.22-9
## [16] ensemblDb_2.36.1
## [17] MASS_7.3-65
## [18] scribe_1.3.7
## [19] backports_1.5.1
## [20] magrittr_2.0.5
## [21] limma_3.68.4
## [22] Hmisc_5.2-5
## [23] rmarkdown_2.31
## [24] yaml_2.3.12
## [25] otl_0.2.0
## [26] doRNG_1.8.6.3
## [27] askpass_1.2.1
## [28] Gviz_1.56.0
## [29] DBI_1.3.0
## [30] RColorBrewer_1.1-3
## [31] abind_1.4-8
## [32] quadprog_1.5-8
## [33] purrr_1.2.2
## [34] R.utils_2.13.0
## [35] AnnotationFilter_1.36.0
## [36] biovizBase_1.60.0
## [37] RCurl_1.98-1.19
## [38] nnet_7.3-20
## [39] VariantAnnotation_1.58.0
## [40] rappdirs_0.3.4
## [41] rentrez_1.2.4
## [42] genefilter_1.94.0
## [43] annotate_1.90.0
## [44] permute_0.9-10
## [45] DelayedMatrixStats_1.34.0
## [46] codetools_0.2-20
## [47] DelayedArray_0.38.2
## [48] xml2_1.5.2
## [49] tidyselect_1.2.1
## [50] UCSC.utils_1.8.0
## [51] farver_2.1.2
```

```
## [52] beanplot_1.3.1
## [53] base64enc_0.1-6
## [54] illuminaio_0.54.0
## [55] GenomicAlignments_1.48.0
## [56] jsonlite_2.0.0
## [57] multtest_2.68.0
## [58] Formula_1.2-5
## [59] survival_3.8-6
## [60] missMethyl_1.46.0
## [61] tools_4.6.0
## [62] progress_1.2.3
## [63] Rcpp_1.1.1-1.1
## [64] glue_1.8.1
## [65] gridExtra_2.3
## [66] SparseArray_1.12.2
## [67] xfun_0.58
## [68] GenomeInfoDb_1.48.0
## [69] dplyr_1.2.1
## [70] HDF5Array_1.40.0
## [71] withr_3.0.2
## [72] IlluminaHumanMethylation450kanno.ilmn12.hg19_0.6.1
## [73] BiocManager_1.30.27
## [74] fastmap_1.2.0
## [75] latticeExtra_0.6-31
## [76] rhdf5filters_1.24.0
## [77] openssl_2.4.2
## [78] digest_0.6.39
## [79] R6_2.6.1
## [80] colorspace_2.1-2
## [81] gtools_3.9.5
## [82] jpeg_0.1-11
## [83] dichromat_2.0-0.1
## [84] biomaRt_2.68.0
## [85] RSQLite_3.53.1
## [86] cigarillo_1.2.0
## [87] R.methodsS3_1.8.2
## [88] h5mread_1.4.0
## [89] tidyr_1.3.2
## [90] data.table_1.18.4
## [91] rtracklayer_1.72.0
## [92] prettyunits_1.2.0
## [93] httr_1.4.8
## [94] htmlwidgets_1.6.4
## [95] S4Arrays_1.12.0
## [96] pkgconfig_2.0.3
```

```
## [97] gtable_0.3.6
## [98] blob_1.3.0
## [99] S7_0.2.2
## [100] siggenes_1.86.0
## [101] htmltools_0.5.9
## [102] ProtGenerics_1.44.0
## [103] scales_1.4.0
## [104] png_0.1-9
## [105] knitr_1.51
## [106] rstudioapi_0.18.0
## [107] tzdb_0.5.0
## [108] rjson_0.2.23
## [109] nlme_3.1-169
## [110] checkmate_2.3.4
## [111] curl_7.1.0
## [112] org.Hs.eg.db_3.23.1
## [113] cachem_1.1.0
## [114] rhdf5_2.56.0
## [115] stringr_1.6.0
## [116] BiocVersion_3.23.1
## [117] foreign_0.8-91
## [118] AnnotationDbi_1.74.0
## [119] restfulr_0.0.16
## [120] GEOquery_2.80.0
## [121] pillar_1.11.1
## [122] grid_4.6.0
## [123] reshape_0.8.10
## [124] vctrs_0.7.3
## [125] beachmat_2.28.0
## [126] xtable_1.8-8
## [127] cluster_2.1.8.2
## [128] htmlTable_2.5.0
## [129] evaluate_1.0.5
## [130] bsseq_1.48.0
## [131] readr_2.2.0
## [132] GenomicFeatures_1.64.0
## [133] cli_3.6.6
## [134] compiler_4.6.0
## [135] Rsamtools_2.28.0
## [136] rngtools_1.5.2
## [137] rlang_1.2.0
## [138] crayon_1.5.3
## [139] nor1mix_1.3-3
## [140] mclust_6.1.2
## [141] interp_1.1-6
```

```
## [142] plyr_1.8.9
## [143] stringi_1.8.7
## [144] deldir_2.0-4
## [145] BiocParallel_1.46.0
## [146] lazyeval_0.2.3
## [147] Matrix_1.7-5
## [148] BSgenome_1.80.0
## [149] hms_1.1.4
## [150] sparseMatrixStats_1.24.0
## [151] bit64_4.8.2
## [152] ggplot2_4.0.3
## [153] Rhdf5lib_2.0.0
## [154] KEGGREST_1.52.0
## [155] statmod_1.5.2
## [156] highr_0.12
## [157] memoise_2.0.1
## [158] bit_4.6.0
## [159] readxl_1.5.0
```

References

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