Introduction to coMethDMR

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`coMethDMR` is an R package that identifies genomic regions that are both co-methylated and differentially methylated in Illumina array datasets. Instead of testing all CpGs within a genomic region, `coMethDMR` carries out an additional step that selects co-methylated sub-regions first without using any outcome information. Next, `coMethDMR` tests association between methylation within the sub-region and continuous phenotype using a random coefficient mixed effects model, which models both variations between CpG sites within the region and differential methylation simultaneously. `coMethDMR` is available from GitHub, and will be submitted to Bioconductor soon.

1. Quick start

1.1 Installation

The latest version can be installed by

```
library(devtools)
install_github ("lissettegomez/coMethDMR")
```

After installation, the `coMethDMR` package can be loaded into R using:

```
library(coMethDMR)
```

1.2 Datasets

The input of `coMethDMR` are methylation beta values. We assume quality control and normalization of the methylation dataset have been performed, by R packages such as `minfi` or `RnBeads`. For illustration, we use a subset of prefrontal cortex methylation data (GEO GSE59685) from a recent Alzheimer's disease epigenome-wide association study which was described in Lunnon et al. (2014). This example dataset contains beta values for 8552 CpGs on chromosome 22 for a random selection of 20 subjects.

```
data(betaMatrixChr22_df)
betaMatrixChr22_df [1:5, 1:5]
```

```
##            GSM1443279 GSM1443663 GSM1443434 GSM1443547 GSM1443577
## cg00004192  0.9249942  0.8463296  0.8700718  0.9058205  0.9090382
## cg00004775  0.6523025  0.6247554  0.7573476  0.6590817  0.6726261
## cg00012194  0.8676339  0.8679048  0.8484754  0.8754985  0.8484458
## cg00013618  0.9466056  0.9475467  0.9566493  0.9588431  0.9419563
## cg00014104  0.3932388  0.5525716  0.4075900  0.3997278  0.3216956
```

The corresponding phenotype dataset included variables `stage` (Braak AD stage), `subject.id`, `Mplate` (batch effect), `Sex`, `Sample` and `age.brain` (age of the brain donor).

```
data(pheno_df)
head(pheno_df)
```

```
##    stage subject.id      Mplate         sex     Sample age.brain
## 3      0          1  6042316048 Sex: FEMALE GSM1443251        82
```

```
## 8      2          2  6042316066 Sex: FEMALE GSM1443256        82
## 10    NA          3  6042316066   Sex: MALE GSM1443258        89
## 15     1          4  7786923107 Sex: FEMALE GSM1443263        81
## 21     2          5  6042316121 Sex: FEMALE GSM1443269        92
## 22     1          6  6042316099   Sex: MALE GSM1443270        78
```

1.3 Vanilla analysis

We are interested in identifying co-methylated genomic regions associated with AD stages (`stage` treated as a linear variable). Here we illustrate analysis of genomic regions mapped to CpG islands, however the workflow can be similarly conducted for other types of genomic region as well. See section 2.1 below.

There are several steps: (1) obtain CpGs located closely (see details in Section 2.1 below) in genomic regions mapped to CpG islands, (2) identify co-methylated regions, and (3) test co-methylated regions against the outcome variable AD stage.

For the first step, we use the following commands:

```
CpGisland_ls <- readRDS(
  system.file (
    "extdata",
    "CpGislandsChr22_ex.RDS",
    package = 'coMethDMR',
    mustWork = TRUE
    )
)
```

Here, `CpGisland_ls` is a list of 20 items, with each item of the list including a group of CpG probe IDs located closely within a particular CpG island region. Section 2.1 discusses how to import additional types of genomic regions.

Next, we identify co-methylated regions:

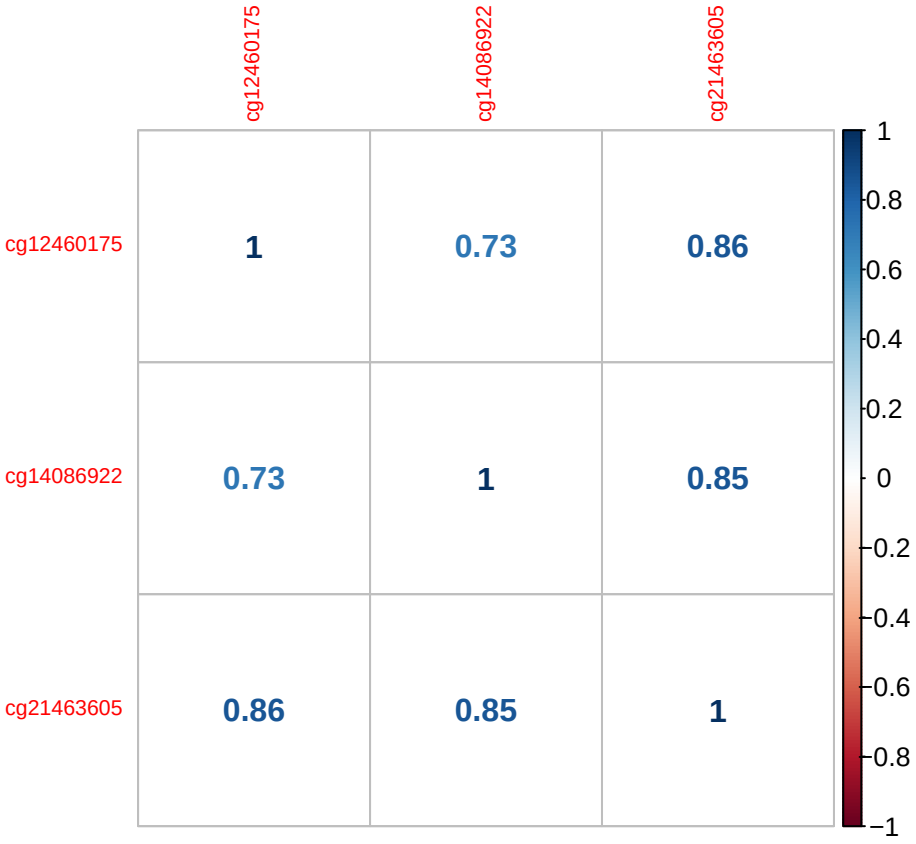
```
coMeth_ls <- CoMethAllRegions (
  betaMatrix = betaMatrixChr22_df,
  betaToM = TRUE,
  CpGs_ls = CpGisland_ls,
  arrayType = "450k",
  returnAllCpGs = FALSE,
  output = "CpGs"
)

coMeth_ls
```

```
## $`chr22:18268062-18268249`
## [1] "cg12460175" "cg14086922" "cg21463605"
##
## $`chr22:18324579-18324769`
## [1] "cg19606103" "cg14031491" "cg03816851"
##
## $`chr22:18531243-18531447`
## [1] "cg25257671" "cg06961233" "cg08819022"
```

`coMeth_ls` is list with that contains groups of CpG probeIDs corresponding to co-methylated regions. Three comethylated regions were identified in this example.

If we want to look at co-methylation within the first co-methylated region:

```r
WriteCorrPlot <- function (beta_mat){

  require (corrplot)
  require (coMethDMR)

  CpGs_char <- row.names (beta_mat)

  CpGsOrd_df <- OrderCpGsByLocation(
    CpGs_char, arrayType=c("450k"), output = "dataframe"
  )

  betaOrdered_mat <- t(beta_mat [CpGsOrd_df$cpg ,])

  corr <- cor (
    betaOrdered_mat, method = "spearman", use = "pairwise.complete.obs"
  )

  corrplot(corr, method="number", number.cex = 1, tl.cex = 0.7)
}

# subsetting beta values to include only co-methylated probes
betas_df <- subset(
  betaMatrixChr22_df,
  row.names(betaMatrixChr22_df) %in% coMeth_ls [[1]]
)

WriteCorrPlot (betas_df)
```



Next, we test these co-methylated regions against `stage` using a random coefficient model (more details in section 2.3 below), adjusting for `age.brain`.

Some messages are generated during mixed models fitting. The interpretations of these messages can be found in the FAQs at the end of this document (see Section 3, item (1) and (2)).

```
out_df <- lmmTestAllRegions(
   beta_df = betaMatrixChr22_df,
   region_ls = coMeth_ls,
   pheno_df,
   contPheno_char = "stage",
   covariates_char = "age.brain",
   modelType = "randCoef",
   arrayType = "450k"
)
```

```
## Fitting linear mixed model to all genomic regions...
## Computation started at 2019-08-03 12:23:40.
##
## [1] "Analyzing region chr22:18268062-18268249"
## [1] "Analyzing region chr22:18324579-18324769"
## [1] "Analyzing region chr22:18531243-18531447"
##
## Computation completed at 2019-08-03 12:23:41.
## Note:
## When mixed model failed to converge, p-value for mixed model is set to 1.
```

```
out_df
```

```
##   chrom    start      end nCpGs    Estimate     StdErr      Stat
## 1 chr22 18268062 18268249     3 -0.07320788 0.03943577 -1.856383
## 2 chr22 18324579 18324769     3  0.03061423 0.02953080  1.036688
## 3 chr22 18531243 18531447     3 -0.07170046 0.04631595 -1.548073
##       pValue       FDR
## 1 0.06339902 0.1824072
## 2 0.29988117 0.2998812
## 3 0.12160480 0.1824072
```

Here `out_df` is a data frame of genomic regions, with corresponding p-values and false discovery rate (FDRs) from the random coefficient mixed model.

We can annotate these results by adding corresponding genes and probes mapped to the genomic regions.

```
outAnno_df <- AnnotateResults(
  lmmRes_df = out_df,
  arrayType = "450k"
)

outAnno_df
```

```
##   chrom    start      end nCpGs    Estimate     StdErr      Stat
## 1 chr22 18268062 18268249     3 -0.07320788 0.03943577 -1.856383
## 2 chr22 18324579 18324769     3  0.03061423 0.02953080  1.036688
## 3 chr22 18531243 18531447     3 -0.07170046 0.04631595 -1.548073
##       pValue       FDR UCSC_RefGene_Group UCSC_RefGene_Accession
## 1 0.06339902 0.1824072
## 2 0.29988117 0.2998812               Body NM_001122731;NM_015241
## 3 0.12160480 0.1824072
##   UCSC_RefGene_Name                            probes
## 1                   cg12460175;cg14086922;cg21463605
## 2            MICAL3 cg03816851;cg14031491;cg19606103
## 3                   cg08819022;cg25257671;cg06961233
```

To further examine the significant regions, we can also extract individual CpG p-values within these significant regions. For example, for the most significant region `chr22:18268062-18268249`,

```
outCpGs_df <- CpGsInfoOneRegion(
 regionName_char = "chr22:18268062-18268249",
 betas_df = betaMatrixChr22_df,
 pheno_df, contPheno_char = "stage",
 covariates_char = "age.brain",
 arrayType = "450k"
)

outCpGs_df
```

```
##                    Region        cpg   chr      pos slopeEstimate
## 1 chr22:18268062-18268249 cg12460175 chr22 18268062       -0.0387
## 2 chr22:18268062-18268249 cg14086922 chr22 18268239       -0.0795
## 3 chr22:18268062-18268249 cg21463605 chr22 18268249       -0.1015
##   slopePval UCSC_RefGene_Name UCSC_RefGene_Accession UCSC_RefGene_Group
## 1    0.3152
## 2    0.0504
## 3    0.0214
```

These CpGs mapped to intergenic regions, so there are no gene names associated with the probes. For genic regions such as `chr22:19709548-19709755`, we would have results such as the following:

```
CpGsInfoOneRegion(
  regionName_char = "chr22:19709548-19709755",
  betas_df = betaMatrixChr22_df,
  pheno_df, contPheno_char = "stage",
  covariates_char = "age.brain",
  arrayType = "450k"
)
```

```
##                    Region        cpg   chr      pos slopeEstimate
## 1 chr22:19709548-19709755 cg04533276 chr22 19709548       -0.0731
## 2 chr22:19709548-19709755 cg20193802 chr22 19709696       -0.0554
## 3 chr22:19709548-19709755 cg05726109 chr22 19709755       -0.0111
##   slopePval UCSC_RefGene_Name UCSC_RefGene_Accession UCSC_RefGene_Group
## 1    0.1229             SEPT5              NM_002688               Body
## 2    0.2097       SEPT5;GP1BB    NM_002688;NM_000407       Body;TSS1500
## 3    0.7714       SEPT5;GP1BB    NM_002688;NM_000407       Body;TSS1500
```

2. Details of the coMethDMR workflow

2.1 Genomic regions

Genomic regions on the Illumina arrays can be defined based on their relations to genes or CpG Islands. Genomic regions related to genes include TSS1500, TSS200, UTR5, EXON1, GENEBODY and UTR3. Genomic regions related to CGIs are NSHORE, NSHELF, ISLAND, SSHORE and SSHELF.

In `coMethDMR` package, the probe IDs for CpGs within each genomic region, as defined by Illumnina 450K array annotation, were obtained from https://rforge.net/IMA/, under "Annotation file". From this list of pre-defined genomic regions, using the function `WriteCloseByAllRegions`, we identified clusters of CpGs located closely (i.e. the maximum separation between any two consecutive probes is 200bp; `maxGap = 200`), and we required each cluster to have at least 3 CpGs (`minCpGs = 3`).

These pre-computed genomic regions can be accessed from our GitHub repository `coMethDMRdata`. For each genomic region, a file with these clusters was created and saved with the suffix '3_200'. For example, to download genomic regions with cluster of CpGs mapped to CpG islands, we use the following commands:

```
gitHubPath_char <- "https://raw.githubusercontent.com/lissettegomez/coMethDMRdata/master/"

CpGisland_ls <- readRDS (
  url(paste0(gitHubPath_char, "ISLAND_3_200.rds"))
)
```

Here `CpGisland_ls` is a list, with each item containing a character vector of CpGs IDs for a particular CpG island.

Similarly, CpGs in other types of genomic regions such as TSS200 can be extracted using the following commands:

```
TSS200_ls <- readRDS (
  url(paste0(gitHubPath_char, "TSS200_3_200.rds"))
)
```

In addition, customized genomic regions can be specified in a .gmt file (http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats#GMT:_Gene_Matrix_Transposed_file_format_

.28.2A.gmt.29) and read into coMethDMR using the read_gmt function.

```
# library(devtools)
# install_github ("gabrielodom/pathwayPCA")
library(pathwayPCA)

TSS200_ls <- read_gmt (
  url(paste0(gitHubPath_char, "TSS200_3_200.gmt")),
  description = FALSE,
  setType = "regions"
)

names(TSS200_ls$regions) <- TSS200_ls$TERMS
```

To extract clusters of close-by CpGs from pre-defined genomic regions with different values of maxGap and minCpGs, the WriteCloseByAllRegions function can be used.

2.2 Identify co-methylated regions

Within each genomic region, we next identify contiguous and co-methylated CpGs sub-regions without using any outcome information. To select these co-methylated sub-regions, we use the rdrop statistic, which is the correlation between each CpG with the sum of methylation levels in all other CpGs. The default is rDropThresh_num = 0.4. We recommend this setting based on our simulation study. Note that higher rDropThresh_num values lead to fewer co-methylated regions.

For example, if we are interested in identifying co-methylated sub-region within the first genomic region in Cgi_ls:

```
Cgi_ls <- readRDS(
  system.file (
    "extdata",
    "CpGislandsChr22_ex.RDS",
    package = 'coMethDMR',
    mustWork = TRUE
    )
)

coMeth_ls <- CoMethAllRegions (
  betaMatrix = betaMatrixChr22_df,
  betaToM = TRUE,
  CpGs_ls = Cgi_ls[1],
  arrayType = "450k",
  returnAllCpGs = FALSE,
  output = "CpGs"
)

coMeth_ls
```

```
## NULL
```

The results indicate there is no co-methylated sub-region within the first genomic region.

Next we look at a region (13th region in Cgi_ls) where there is a co-methylated sub-region:

```
coMeth_ls <- CoMethAllRegions (
  betaMatrix = betaMatrixChr22_df,
```

```
  betaToM = TRUE,
  CpGs_ls = Cgi_ls[13],
  arrayType = "450k",
  returnAllCpGs = FALSE,
  output = "CpGs"
)

coMeth_ls
```

```
## $`chr22:18268062-18268249`
## [1] "cg12460175" "cg14086922" "cg21463605"
```

`coMeth_ls` is a list, where each item is a list of CpG probe IDs for a co-methylated sub-region.

If we want to see the detailed output of the coMethDMR algorithm, that is, how the co-methylated region was obtained, we can specify `output = "dataframe"`:

```
coMethData_ls <- CoMethAllRegions (
  betaMatrix = betaMatrixChr22_df,
  betaToM = TRUE,
  CpGs_ls = Cgi_ls[13],
  arrayType = "450k",
  returnAllCpGs = FALSE,
  output = "dataframe"
)

coMethData_ls
```

```
## $`chr22:18267969-18268249`
##                    Region        CpG   Chr  MAPINFO    r_drop keep
## 1 chr22:18267969-18268249 cg18370151 chr22 18267969 0.3939650    0
## 2 chr22:18267969-18268249 cg12460175 chr22 18268062 0.8341630    1
## 3 chr22:18267969-18268249 cg14086922 chr22 18268239 0.7918945    1
## 4 chr22:18267969-18268249 cg21463605 chr22 18268249 0.8604731    1
##   keep_contiguous
## 1               0
## 2               1
## 3               1
## 4               1
```

`coMethData_ls` provides the details on how the co-methylated region was obtained: Here `keep = 1` if `rDropThresh_num > 0.4` (i.e. a co-methylated CpG), and `keep_contigous` indicates if the probe is in a contiguous co-methylated region.

2.3 Testing genomic regions against a continuous phenotype

To test association between a continuous phenotype and methylation values in a contiguous co-methylated region, two mixed models have been implemented in the function `lmmTestAllRegions`: a random coefficient mixed model (`modelType = "randCoef"`) and a simple linear mixed model (`modelType = "simple"`).

The random coefficient mixed model includes both a systematic component that models the mean for each group of CpGs, and a random component that models how each CpG varies with respect to the group mean (random probe effects). It also includes random sample effects that model correlations between multiple probes within the same sample.

More specifically, the random coefficient model is `methylation M value ~ contPheno_char +`

`covariates_char + (1|Sample) + (contPheno_char|CpG)`. The last term `(contPheno_char|CpG)` specifies both random intercepts and slopes for each CpG.

The simple linear mixed model includes all the terms in the random coefficient model except random probe effects.

The simple linear mixed model is

`methylation M value ~ contPheno_char + covariates_char + (1|Sample)`

To test one genomic region against the continuous phenotype `stage`, adjusting for `age.brain`:

```
lmmTestAllRegions(
  beta_df = betaMatrixChr22_df,
  region_ls = coMeth_ls[1],
  pheno_df,
  contPheno_char = "stage",
  covariates_char = "age.brain",
  modelType = "randCoef",
  arrayType = "450k"
)
```

```
## Fitting linear mixed model to all genomic regions...
## Computation started at 2019-08-03 12:23:46.
##
## [1] "Analyzing region chr22:18268062-18268249"
##
## Computation completed at 2019-08-03 12:23:46.
## Note:
## When mixed model failed to converge, p-value for mixed model is set to 1.
```

```
##   chrom    start      end nCpGs    Estimate     StdErr      Stat
## 1 chr22 18268062 18268249     3 -0.07320788 0.03943577 -1.856383
##       pValue        FDR
## 1 0.06339902 0.06339902
```

If we don't want to adjust for any covariate effect, we can set `covariates_char` to `NULL`:

```
lmmTestAllRegions(
  beta_df = betaMatrixChr22_df,
  region_ls = coMeth_ls[1],
  pheno_df,
  contPheno_char = "stage",
  covariates_char = NULL,
  modelType = "randCoef",
  arrayType = "450k"
)
```

```
## Fitting linear mixed model to all genomic regions...
## Computation started at 2019-08-03 12:23:46.
##
## [1] "Analyzing region chr22:18268062-18268249"
##
## Computation completed at 2019-08-03 12:23:46.
## Note:
## When mixed model failed to converge, p-value for mixed model is set to 1.
```

```
##   chrom    start      end nCpGs    Estimate     StdErr     Stat     pValue
## 1 chr22 18268062 18268249     3 -0.06678558 0.03883719 -1.71963 0.08549977
```

```
##          FDR
## 1 0.08549977
```

3. Frequently Asked Questions

(1) What happens when mixed model fails to coverge (i.e. the warning "Model failed to converge with. . . " is resulted for a particular genomic region)?

- In this case, the p-value for mixed model is set to 1. In our experiences with methylation datasets, genomic regions with strong signals typically converge. Convergence issues typically occurs when the amount of noise in data is high.

(2) When fitting mixed models with `lmmTestAllRegions` function, What does the message "boundary (singular) fit" mean?

- When mixed model is singular, at least one of the estimated variance components for intercepts or slopes random effects is 0, because there isn't enough variabilities in data to estimate the random effects. In this case, mixed model reduces to a fixed effects model. However, as our simulation studies have shown, the p-values obtained for these regions are still valid.

4. Reference

Lunnon K, Smith R, Hannon E, De Jager PL, Srivastava G, Volta M, Troakes C, Al-Sarraj S, Burrage J, Macdonald R, et al (2014) Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. Nat Neurosci 17:1164-1170.