

# HIREewas: Detection of Cell-Type-Specific Risk-CpG Sites in EWAS

## User's Guide

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# HIREewas: Detection of Cell-Type-Specific Risk-CpG Sites in EWAS

## User's Guide

### 1 Introduction

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In epigenome-wide association studies (EWAS), as samples are measured at the bulk level rather than at the single-cell level, the obtained methylome for each sample shows the signals aggregated from distinct cell types [1, 2, 3]. The cellular heterogeneity leads to two main challenges for analyzing EWAS data.

On the one hand, the cell type compositions differ between samples and can be associated with phenotypes [1, 3]. Both binary phenotypes, such as the diseased or normal status [1], and continuous phenotypes, for example, age [3], have been found to affect the cell type compositions. As a result, ignoring the cellular heterogeneity in EWAS can lead to a large number of spurious associations [3, 4, 5, 6]. On the other hand, the phenotype may change the methylation level of a CpG site in some but not all of the cell types. Identifying the exact cell types that carry the risk-CpG sites can deepen our understandings of disease mechanisms. Nevertheless, such identification is challenging because we can only observe the aggregated-level signals.

However, to the best of our knowledge, no existing statistical method for EWAS can detect cell-type-specific associations despite the active research on accounting for cell-type heterogeneity. The existing approaches can be categorized into two schools [7]: “reference-based” and “reference-free” methods. As the method names indicate, the reference-based methods [2, 8] require the reference methylation profiles for each cell type to be known a priori, while the reference-free methods do not depend on any known methylation reference by employing matrix decomposition techniques [9] or extracting surrogate variables including principle components as a special case [10, 11, 6, 4].

Although all of the existing methods aim to address the cellular heterogeneity problem in EWAS and claim whether a CpG site is associated with phenotypes at the *aggregated level*, none of them can identify the risk-CpG sites for each *individual cell type*, thus missing the opportunity to obtain finer-grained results in EWAS.

We propose a hierarchical model HIRE [?] to identify the association in EWAS at an unprecedented High REsolution: detecting whether a CpG site has any associations with the phenotypes in each cell type. HIRE not only substantially improves the power of association detection at the aggregated level as compared to the existing methods but also enables the

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detection of risk-CpG sites for individual cell types. HIRE is applicable to EWAS with binary phenotypes, continuous phenotypes, or both.

The user's guide provides step-by-step instructions for the *HIREewas* R package. We believe that, by helping biology researchers understand in which cell types the CpG sites are affected by a disease using *HIREewas*, HIRE can ultimately facilitate the development of epigenetic therapies by targeting the specifically affected cell types.

## 2 Data Preparation

---

We first introduce the input data format. The input data consists of the methylation values and the covariates. The methylation values should be organized into a matrix `0meth`, where each row represents a CpG site and each column corresponds to a sample. In other words, the  $(i, j)$  element of `0meth` is the methylation value for sample  $j$  in CpG site  $i$ . The covariate data are also arranged in a matrix `X`. Each row of `X` denotes one covariate, so the row number is equal to the number of covariate types. Columns of `X` represent samples. Therefore, the  $(\ell, j)$  element of `X` is the covariate  $\ell$  information of sample  $j$ .

For demonstration, we then generate a dataset following the simulation steps in [?].

```
#####  
#Generate the EWAS data  
#####  
  
set.seed(123)  
###define a function to draw samples from a Dirichlet distribution  
rDirichlet <- function(alpha_vec){  
  num <- length(alpha_vec)  
  temp <- rgamma(num, shape = alpha_vec, rate = 1)  
  return(temp / sum(temp))  
}  
  
n <- 180      #number of samples  
n1 <- 60     #number of controls
```

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```
n2 <- 120    #number of cases

m <- 2000    #number of CpG sites
K <- 3      #underlying cell type number

###simulate methylation baseline profiles
#assume cell type 1 and cell type 2 are from the same lineage
#cell type 1
methy1 <- rbeta(m,3,6)
#cell type 2
methy2 <- methy1 + rnorm(m, sd=0.01)
ind <- sample(1:m, m/5)
methy2[ind] <- rbeta(length(ind),3,6)

#cell type 3
methy3 <- rbeta(m,3,6)
mu <- cbind(methy1, methy2, methy3)

#number of covariates
p <- 2

###simulate covariates / phenotype (disease status and age)
X <- rbind(c(rep(0, n1),rep(1, n2)), runif(n, min=20, max=50))

###simulate phenotype effects
beta <- array(0, dim=c(m,K,p))

#control vs case
m_common <- 10
max_signal <- 0.15
min_signal <- 0.07

#we allow different signs and magnitudes
signs <- sample(c(-1,1), m_common*K, replace=TRUE)
beta[1:m_common,1:K,1] <- signs * runif(m_common*K, min=min_signal, max=max_signal)

m_seperate <- 10
signs <- sample(c(-1,1), m_seperate*2, replace=TRUE)
```

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```
beta[m_common+(1:m_seperate),1:2,1] <- signs *
                                runif(m_seperate*2, min=min_signal, max=max_signal)

signs <- sample(c(-1,1), m_seperate, replace=TRUE)
beta[m_common+m_seperate+(1:m_seperate),K,1] <- signs *
                                runif(m_seperate, min=min_signal, max=max_signal)

#age
base <- 20
m_common <- 10
max_signal <- 0.015
min_signal <- 0.007
signs <- sample(c(-1,1), m_common*K, replace=TRUE)
beta[base+1:m_common,1:K,2] <- signs *
                                runif(m_common*K, min=min_signal, max=max_signal)

m_seperate <- 10
signs <- sample(c(-1,1), m_seperate*2, replace=TRUE)
beta[base+m_common+(1:m_seperate),1:2,2] <- signs *
                                runif(m_seperate*2, min=min_signal, max=max_signal)

signs <- sample(c(-1,1), m_seperate, replace=TRUE)
beta[base+m_common+m_seperate+(1:m_seperate),K,2] <- signs *
                                runif(m_seperate, min=min_signal, max=max_signal)

###generate the cellular compositions
P <- sapply(1:n, function(i){
                                if(X[1,i]==0){ #if control
                                    rDirichlet(c(4,4, 2+X[2,i]/10))
                                }else{
                                    rDirichlet(c(4,4, 5+X[2,i]/10))
                                }
                            })

###generate the observed methylation profiles
Ometh <- NULL
for(i in 1:n){
    utmp <- t(sapply(1:m, function(j){
```

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```
tmp1 <- colSums(X[ ,i] * t(beta[j, , ]))
          rnorm(K,mean=mu[j, ]+tmp1,sd=0.01)
          })
tmp2 <- colSums(P[ ,i] * t(utmp))
Ometh <- cbind(Ometh, tmp2 + rnorm(m, sd = 0.01))
}

#constrain methylation values between 0 and 1
Ometh[Ometh > 1] <- 1

Ometh[Ometh < 0] <- 0
```

Here we simulated a methylation matrix `Ometh` with 2,000 CpG sites and 180 samples as well as a covariate matrix `X` with one binary covariate (case/control) in the first row and one continuous variable (age) in the second row. We can further look into the details.

```
#the class of the methylation matrix
class(Ometh)

## [1] "matrix" "array"

#the values in the methylation matrix
head(Ometh[,1:6])

##           [,1]      [,2]      [,3]      [,4]      [,5]      [,6]
## [1,] 0.3202140 0.3440603 0.3619823 0.2854539 0.3861594 0.3808383
## [2,] 0.2839342 0.3131437 0.3168782 0.3031998 0.3207638 0.3062112
## [3,] 0.5767125 0.5339113 0.5143695 0.6126050 0.5041764 0.5092417
## [4,] 0.3597017 0.2931941 0.3259443 0.3294055 0.3200657 0.2760185
## [5,] 0.3632531 0.3303706 0.3678126 0.3569870 0.3607428 0.3345626
## [6,] 0.3645631 0.3281156 0.3308156 0.4126270 0.3089588 0.3112898

#the class of the covariate matrix
class(X)

## [1] "matrix" "array"

#the values in the covariate matrix
X[,1:6]

##           [,1]      [,2]      [,3]      [,4]      [,5]      [,6]
```

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```
## [1,] 0.00000 0.00000 0.00000 0.00000 0.0000 0.00000  
## [2,] 30.46081 44.20032 40.51409 25.41305 27.6765 41.67172
```

### 3 Model Application

---

Once we have prepared the data `Ometh` and `X` described before, we can use the R function `HIRE` to carry out the HIRE model in a convenient way.

```
library(HIREewas)  
ret_list <- HIRE(Ometh, X, num_celltype=K, tol=10^(-5), num_iter=1000, alpha=0.01)  
  
## Initialization Done.  
  
## Implementing EM algorithm...  
  
## Iteration: 1 observed-data log likelihood: 786977.047009  
## Iteration: 2 observed-data log likelihood: 845597.548734  
## Iteration: 3 observed-data log likelihood: 890628.061074  
## Iteration: 4 observed-data log likelihood: 928582.504293  
## Iteration: 5 observed-data log likelihood: 961110.843322  
## Iteration: 6 observed-data log likelihood: 988776.683578  
## Iteration: 7 observed-data log likelihood: 1011852.503606  
## Iteration: 8 observed-data log likelihood: 1030600.549852  
## Iteration: 9 observed-data log likelihood: 1045380.540807  
## Iteration: 10 observed-data log likelihood: 1056672.531894  
## Iteration: 11 observed-data log likelihood: 1065043.271409  
## Iteration: 12 observed-data log likelihood: 1071085.718678  
## Iteration: 13 observed-data log likelihood: 1075356.124045  
## Iteration: 14 observed-data log likelihood: 1078337.083847  
## Iteration: 15 observed-data log likelihood: 1080414.137330  
## Iteration: 16 observed-data log likelihood: 1081876.180194  
## Iteration: 17 observed-data log likelihood: 1082929.295159  
## Iteration: 18 observed-data log likelihood: 1083713.865411  
## Iteration: 19 observed-data log likelihood: 1084323.512061  
## Iteration: 20 observed-data log likelihood: 1084818.855742  
## Iteration: 21 observed-data log likelihood: 1085238.175662
```

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```
## Iteration: 22 observed-data log likelihood: 1085605.540460
## Iteration: 23 observed-data log likelihood: 1085936.061253
## Iteration: 24 observed-data log likelihood: 1086239.303418
## Iteration: 25 observed-data log likelihood: 1086521.422498
## Iteration: 26 observed-data log likelihood: 1086786.482879
## Iteration: 27 observed-data log likelihood: 1087037.259105
## Iteration: 28 observed-data log likelihood: 1087275.724644
## Iteration: 29 observed-data log likelihood: 1087503.363960
## Iteration: 30 observed-data log likelihood: 1087721.317017
## Iteration: 31 observed-data log likelihood: 1087930.498253
## Iteration: 32 observed-data log likelihood: 1088131.662261
## Iteration: 33 observed-data log likelihood: 1088325.445237
## Iteration: 34 observed-data log likelihood: 1088512.391991
## Iteration: 35 observed-data log likelihood: 1088692.974167
## Iteration: 36 observed-data log likelihood: 1088867.603854
## Iteration: 37 observed-data log likelihood: 1089036.642900
## Iteration: 38 observed-data log likelihood: 1089200.410566
## Iteration: 39 observed-data log likelihood: 1089359.189746
## Iteration: 40 observed-data log likelihood: 1089513.232186
## Iteration: 41 observed-data log likelihood: 1089662.762934
## Iteration: 42 observed-data log likelihood: 1089807.984191
## Iteration: 43 observed-data log likelihood: 1089949.078641
## Iteration: 44 observed-data log likelihood: 1090086.212307
## Iteration: 45 observed-data log likelihood: 1090219.536998
## Iteration: 46 observed-data log likelihood: 1090349.192376
## Iteration: 47 observed-data log likelihood: 1090475.307698
## Iteration: 48 observed-data log likelihood: 1090598.003281
## Iteration: 49 observed-data log likelihood: 1090717.391728
## Iteration: 50 observed-data log likelihood: 1090833.578968
## Iteration: 51 observed-data log likelihood: 1090946.665130
## Iteration: 52 observed-data log likelihood: 1091056.745286
## Iteration: 53 observed-data log likelihood: 1091163.910086
## Iteration: 54 observed-data log likelihood: 1091268.246292
## Iteration: 55 observed-data log likelihood: 1091369.837229
## Iteration: 56 observed-data log likelihood: 1091468.763175
## Iteration: 57 observed-data log likelihood: 1091565.101671
## Iteration: 58 observed-data log likelihood: 1091658.927794
## Iteration: 59 observed-data log likelihood: 1091750.314375
```



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```
## Iteration: 60 observed-data log likelihood: 1091839.332178
## Iteration: 61 observed-data log likelihood: 1091926.050047
## Iteration: 62 observed-data log likelihood: 1092010.535025
## Iteration: 63 observed-data log likelihood: 1092092.852448
## Iteration: 64 observed-data log likelihood: 1092173.066019
## Iteration: 65 observed-data log likelihood: 1092251.237871
## Iteration: 66 observed-data log likelihood: 1092327.428610
## Iteration: 67 observed-data log likelihood: 1092401.697354
## Iteration: 68 observed-data log likelihood: 1092474.097229
## Iteration: 69 observed-data log likelihood: 1092544.688201
## Iteration: 70 observed-data log likelihood: 1092613.525653
## Iteration: 71 observed-data log likelihood: 1092680.663110
## Iteration: 72 observed-data log likelihood: 1092746.152664
## Iteration: 73 observed-data log likelihood: 1092810.045014
## Iteration: 74 observed-data log likelihood: 1092872.389487
## Iteration: 75 observed-data log likelihood: 1092933.234059
## Iteration: 76 observed-data log likelihood: 1092992.625380
## Iteration: 77 observed-data log likelihood: 1093050.608794
## Iteration: 78 observed-data log likelihood: 1093107.228354
## Iteration: 79 observed-data log likelihood: 1093162.526843
## Iteration: 80 observed-data log likelihood: 1093216.545797
## Iteration: 81 observed-data log likelihood: 1093269.325517
## Iteration: 82 observed-data log likelihood: 1093320.905095
## Iteration: 83 observed-data log likelihood: 1093371.322433
## Iteration: 84 observed-data log likelihood: 1093420.614259
## Iteration: 85 observed-data log likelihood: 1093468.816150
## Iteration: 86 observed-data log likelihood: 1093515.962553
## Iteration: 87 observed-data log likelihood: 1093562.086806
## Iteration: 88 observed-data log likelihood: 1093607.221156
## Iteration: 89 observed-data log likelihood: 1093651.396786
## Iteration: 90 observed-data log likelihood: 1093694.643833
## Iteration: 91 observed-data log likelihood: 1093736.991415
## Iteration: 92 observed-data log likelihood: 1093778.467649
## Iteration: 93 observed-data log likelihood: 1093819.099683
## Iteration: 94 observed-data log likelihood: 1093858.913713
## Iteration: 95 observed-data log likelihood: 1093897.935015
## Iteration: 96 observed-data log likelihood: 1093936.187963
## Iteration: 97 observed-data log likelihood: 1093973.696061
```

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```
## Iteration: 98 observed-data log likelihood: 1094010.481965
## Iteration: 99 observed-data log likelihood: 1094046.567509
## Iteration: 100 observed-data log likelihood: 1094081.973727
## Iteration: 101 observed-data log likelihood: 1094116.720880
## Iteration: 102 observed-data log likelihood: 1094150.828478
## Iteration: 103 observed-data log likelihood: 1094184.315299
## Iteration: 104 observed-data log likelihood: 1094217.199415
## Iteration: 105 observed-data log likelihood: 1094249.498208
## Iteration: 106 observed-data log likelihood: 1094281.228391
## Iteration: 107 observed-data log likelihood: 1094312.406027
## Iteration: 108 observed-data log likelihood: 1094343.046546
## Iteration: 109 observed-data log likelihood: 1094373.164764
## Iteration: 110 observed-data log likelihood: 1094402.774902
## Iteration: 111 observed-data log likelihood: 1094431.890604
## Iteration: 112 observed-data log likelihood: 1094460.524958
## Iteration: 113 observed-data log likelihood: 1094488.690519
## Iteration: 114 observed-data log likelihood: 1094516.399329
## Iteration: 115 observed-data log likelihood: 1094543.662944
## Iteration: 116 observed-data log likelihood: 1094570.492457
## Iteration: 117 observed-data log likelihood: 1094596.898527
## Iteration: 118 observed-data log likelihood: 1094622.891408
## Iteration: 119 observed-data log likelihood: 1094648.480970
## Iteration: 120 observed-data log likelihood: 1094673.676734
## Iteration: 121 observed-data log likelihood: 1094698.487892
## Iteration: 122 observed-data log likelihood: 1094722.923335
## Iteration: 123 observed-data log likelihood: 1094746.991677
## Iteration: 124 observed-data log likelihood: 1094770.701273
## Iteration: 125 observed-data log likelihood: 1094794.060240
## Iteration: 126 observed-data log likelihood: 1094817.075117
## Iteration: 127 observed-data log likelihood: 1094839.753146
## Iteration: 128 observed-data log likelihood: 1094862.101817
## Iteration: 129 observed-data log likelihood: 1094884.128484
## Iteration: 130 observed-data log likelihood: 1094905.840368
## Iteration: 131 observed-data log likelihood: 1094927.244549
## Iteration: 132 observed-data log likelihood: 1094948.347974
## Iteration: 133 observed-data log likelihood: 1094969.157448
## Iteration: 134 observed-data log likelihood: 1094989.679641
## Iteration: 135 observed-data log likelihood: 1095009.921083
```

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```
## Iteration: 136 observed-data log likelihood: 1095029.888172
## Iteration: 137 observed-data log likelihood: 1095049.585515
## Iteration: 138 observed-data log likelihood: 1095069.002001
## Iteration: 139 observed-data log likelihood: 1095088.159459
## Iteration: 140 observed-data log likelihood: 1095107.064927
## Iteration: 141 observed-data log likelihood: 1095125.724658
## Iteration: 142 observed-data log likelihood: 1095144.144466
## Iteration: 143 observed-data log likelihood: 1095162.329879
## Iteration: 144 observed-data log likelihood: 1095180.286223
## Iteration: 145 observed-data log likelihood: 1095198.018669
## Iteration: 146 observed-data log likelihood: 1095215.532251
## Iteration: 147 observed-data log likelihood: 1095232.831881
## Iteration: 148 observed-data log likelihood: 1095249.922348
## Iteration: 149 observed-data log likelihood: 1095266.808323
## Iteration: 150 observed-data log likelihood: 1095283.494359
## Iteration: 151 observed-data log likelihood: 1095299.984889
## Iteration: 152 observed-data log likelihood: 1095316.284229
## Iteration: 153 observed-data log likelihood: 1095332.396576
## Iteration: 154 observed-data log likelihood: 1095348.326012
## Iteration: 155 observed-data log likelihood: 1095364.076505
## Iteration: 156 observed-data log likelihood: 1095379.651909
## Iteration: 157 observed-data log likelihood: 1095395.055968
## Iteration: 158 observed-data log likelihood: 1095410.292316
## Iteration: 159 observed-data log likelihood: 1095425.364480
## Iteration: 160 observed-data log likelihood: 1095440.275884
## Iteration: 161 observed-data log likelihood: 1095455.029347
## Iteration: 162 observed-data log likelihood: 1095469.627390
## Iteration: 163 observed-data log likelihood: 1095484.074210
## Iteration: 164 observed-data log likelihood: 1095498.372868
## Iteration: 165 observed-data log likelihood: 1095512.526315
## Iteration: 166 observed-data log likelihood: 1095526.537406
## Iteration: 167 observed-data log likelihood: 1095540.408905
## Iteration: 168 observed-data log likelihood: 1095554.143492
## Iteration: 169 observed-data log likelihood: 1095567.743767
## Iteration: 170 observed-data log likelihood: 1095581.212253
## Iteration: 171 observed-data log likelihood: 1095594.551396
## Iteration: 172 observed-data log likelihood: 1095607.763570
## Iteration: 173 observed-data log likelihood: 1095620.851077
```

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```
## Iteration: 174 observed-data log likelihood: 1095633.816151
## Iteration: 175 observed-data log likelihood: 1095646.660961
## Iteration: 176 observed-data log likelihood: 1095659.387609
## Iteration: 177 observed-data log likelihood: 1095671.998136
## Iteration: 178 observed-data log likelihood: 1095684.494522
## Iteration: 179 observed-data log likelihood: 1095696.878691
## Iteration: 180 observed-data log likelihood: 1095709.152509
## Iteration: 181 observed-data log likelihood: 1095721.317788
## Iteration: 182 observed-data log likelihood: 1095733.376288
## Iteration: 183 observed-data log likelihood: 1095745.329718
## Iteration: 184 observed-data log likelihood: 1095757.179741
## Iteration: 185 observed-data log likelihood: 1095768.927969
## Iteration: 186 observed-data log likelihood: 1095780.575973
## Iteration: 187 observed-data log likelihood: 1095792.125278
## Iteration: 188 observed-data log likelihood: 1095803.577368
## Iteration: 189 observed-data log likelihood: 1095814.933686
## Iteration: 190 observed-data log likelihood: 1095826.195637
## Iteration: 191 observed-data log likelihood: 1095837.364358
## Iteration: 192 observed-data log likelihood: 1095848.439878
## Iteration: 193 observed-data log likelihood: 1095859.424424
## Iteration: 194 observed-data log likelihood: 1095870.319297

## Done!

## Calculating p-values...

## Done!
```

Among the arguments of the `HIRE`, `num_celltype` is the number of cell types specified by the user, which can be decided by prior knowledge or the penalized BIC criterion [12]. `tol` is the relative tolerance to determine when `HIRE` stops. Specifically, when the ratio of the log observed-data likelihood difference to the log observed-data likelihood at last iteration in the absolute value is smaller than `tol`, then the `HIRE` functions stops. The default is  $10e-5$ . `num_iter` is the maximum number that `HIRE` iterates with default 1000. `alpha` is a threshold parameter used in the Bonferroni correction to claim a significant cell-type-specific CpG site and to calculate the penalized BIC, and its default is 0.01.

The return value `ret_list` is an R list that consists of all parameter estimates of our interest.

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```
# the class of ret_list
class(ret_list)

## [1] "list"

#the estimated cellular compositions
ret_list$P_t[ ,1:6]

##           [,1]      [,2]      [,3]      [,4]      [,5]      [,6]
## [1,] 0.1901593 0.03852461 0.2354742 0.00000000 0.2950168 0.09693634
## [2,] 0.6427212 0.57371963 0.3283484 0.94800234 0.2241097 0.32122424
## [3,] 0.1671195 0.38775577 0.4361773 0.05199766 0.4808736 0.58183942

#the estimated cell-type-specific methylation baseline profiles
head(ret_list$mu_t)

##           [,1]      [,2]      [,3]
## [1,] 0.3750383 0.2229805 0.4659452
## [2,] 0.3238664 0.2794961 0.3271067
## [3,] 0.5034966 0.6631053 0.4279233
## [4,] 0.4161573 0.3685475 0.2394163
## [5,] 0.3904720 0.4048575 0.2589418
## [6,] 0.3655453 0.4189415 0.2260232

#the estimated phenotype effects
head(ret_list$beta_t)

## , , 1
##
##           [,1]      [,2]      [,3]
## [1,] 0.14249392 -0.04557257 0.10353841
## [2,] -0.08631466 -0.09469071 -0.08614096
## [3,] -0.10966325 0.08380155 0.06667019
## [4,] -0.13608425 0.07704315 0.09942264
## [5,] -0.18016851 0.05957414 -0.09013889
## [6,] 0.01040779 0.05899374 -0.09657628
##
## , , 2
##
##           [,1]      [,2]      [,3]
## [1,] -0.0013749510 0.0014330780 -5.426761e-04
```

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```
## [2,] -0.0005231190  0.0005819393  1.439812e-05
## [3,]  0.0017287586 -0.0010754865 -1.679216e-04
## [4,]  0.0016654162 -0.0007056863 -4.924230e-04
## [5,]  0.0014824095 -0.0014435132  5.042827e-04
## [6,]  0.0001832922 -0.0006986387  9.029005e-04

#the penalized BIC value
ret_list$pBIC

## [1] -2116536

#the estimated p-values to claim whether a CpG site is at risk
#in some cell type for a covariate

#p value matrix for case/control
head(ret_list$pvalues[ ,1:3])

##           x_matr1      x_matr2      x_matr3
## [1,] 1.548811e-27 1.340803e-11 4.383043e-36
## [2,] 3.684765e-09 3.898512e-26 1.837266e-29
## [3,] 1.638936e-15 5.006148e-22 4.659029e-20
## [4,] 1.945476e-18 3.138753e-18 6.131076e-32
## [5,] 5.492153e-34 3.812436e-16 2.041123e-35
## [6,] 5.732404e-01 9.423626e-14 5.271416e-36

#p value matrix for age
head(ret_list$pvalues[ ,4:6])

##           x_matr4      x_matr5      x_matr6
## [1,] 0.9309509 0.8547646 0.4038134
## [2,] 0.7983960 0.9283010 0.4595236
## [3,] 0.9590815 0.7141966 0.8647336
## [4,] 0.1197502 0.3686029 0.7868304
## [5,] 0.6731682 0.9416187 0.9164448
## [6,] 0.8241503 0.8953657 0.1928233
```

`ret_list$P_t` is the estimated cell proportion matrix with its rows denoting cell types and columns representing samples. We can also compare the estimates with the underlying truth (see the following code). Since the deconvolution technique is unsupervised, the label-switching problem exists. Therefore, we use (2,1,3) to index `ret_list$P_t` instead of (1,2,3). `ret_list$mu_t` is the estimated cell-type-specific methylation baselines in a matrix form, where

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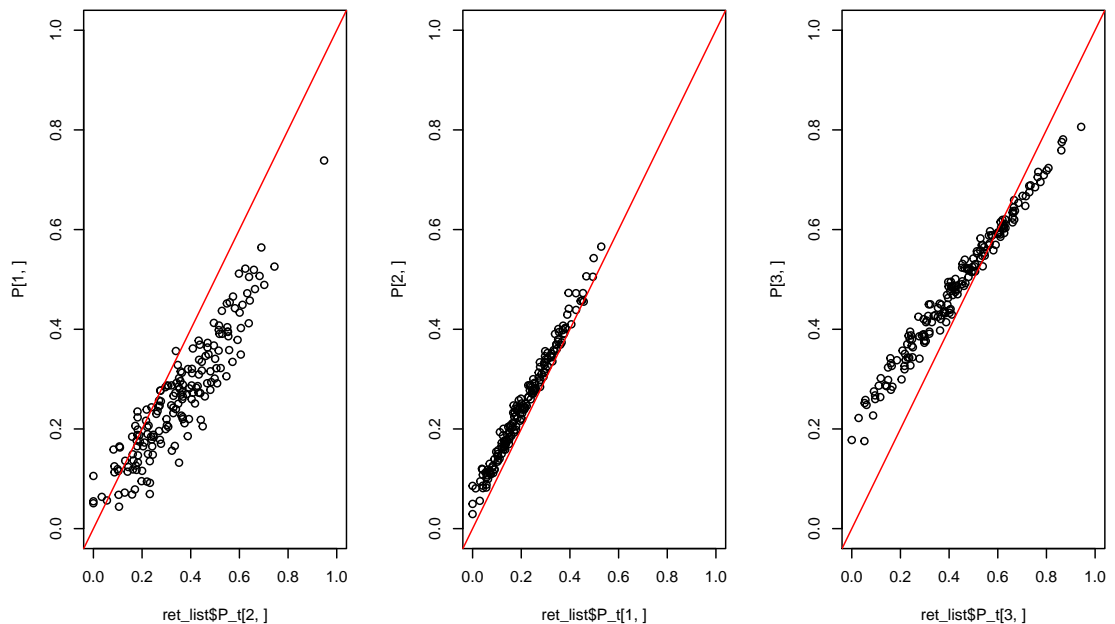
CpG sites in rows and cell types in column. `ret_list$beta_t` is a three dimensional array where `ret_list$beta_t[i,k,ell]` is the estimated phenotype `ell` effect on CpG site `i` in cell type `k`. The penalized BIC score can be obtained by `ret_list$pBIC`.

The approximate p values are stored in the matrix `ret_list$pvalues`. Its dimension is `m` (the CpG site number) by `Kq` (the cell type number `K` times the phenotype number `q`). In the p-value matrix, one row is a CpG site. The first `K` columns correspond to the p-value matrix of the phenotype 1, the second `K` columns corresponds to the p-value matrix of the phenotype 2, and so forth.

```
#estimated cell compositions vs the truth
par(mfrow=c(1,3))
plot(ret_list$P_t[2, ], P[1, ], xlim=c(0,1), ylim=c(0,1))
abline(a=0, b=1, col="red")

plot(ret_list$P_t[1, ], P[2, ], xlim=c(0,1), ylim=c(0,1))
abline(a=0, b=1, col="red")

plot(ret_list$P_t[3, ], P[3, ], xlim=c(0,1), ylim=c(0,1))
abline(a=0, b=1, col="red")
```

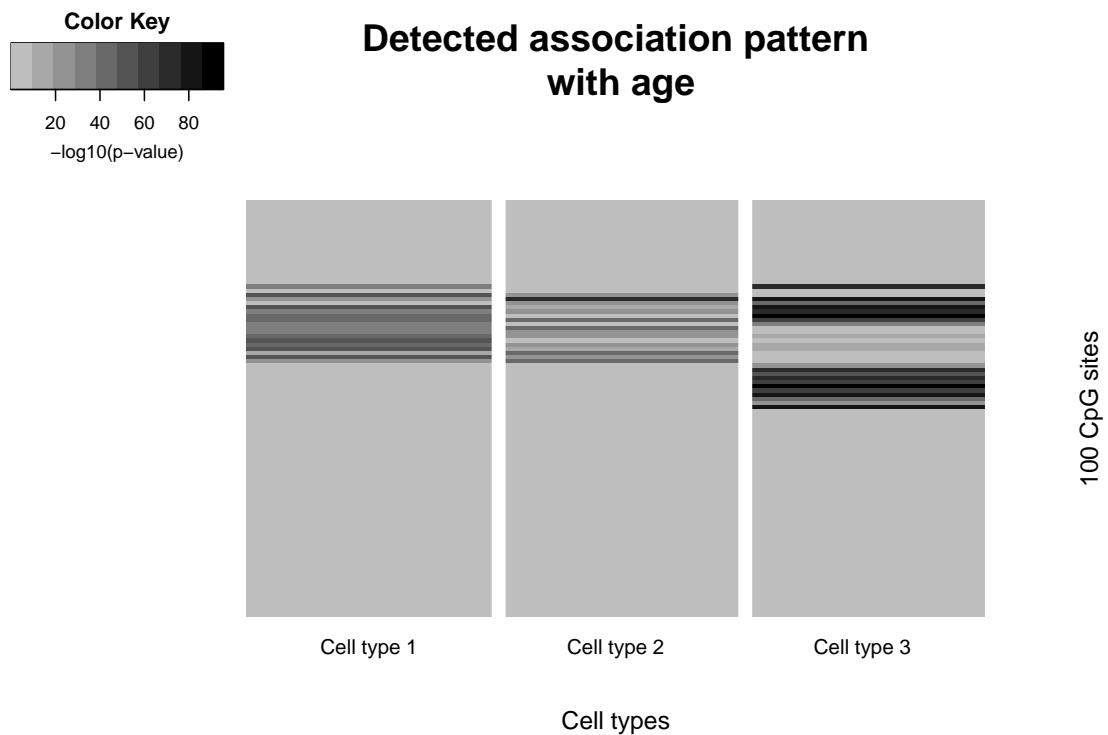


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## 4 Visualization

We can visualize the detected association in a cell-type-specific way using `riskCpGpattern` as follows.

```
riskCpGpattern(ret_list$pvalues[1:100, K+c(2,1,3)],  
              main_title="Detected association pattern\n with age", hc_row_ind = FALSE)
```



Here, for a good visualization, only the p-values for the first 100 CpG sites were demonstrated. `main_title` is used to specify the title of the association figure. `hc_row_ind` is an argument indicating whether the rows should be hierarchically clustered.



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