epimutacions a Bioconductor package to identify outliers in rare diseases DNA methylation data

Supplementary material

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Contents

1 Introduction

1.1 Background

Rare diseases are pathologies with a low prevalence (< 1 per 2,000 people) (European-Commission 2020). Most of these pathologies have an onset during childhood and a strong genetic etiology (López-Bastida et al. 2016). Consequently, rare disease diagnosis has relied on identifying genetic and genomic mutations that can cause the disease (Aref-Eshghi et al. 2019). Although these variants have provided a diagnosis for many patients and families, around 60% of the cases remained undiagnosed (Lionel et al. 2018). Aberrant methylation can be an underlying cause of undiagnosed patients, either as a primary event (a.k.a. epimutation) or as a functional consequence of chromatin dysregulation by genetic or environmental agents (a.k.a. episignature) (Aref-Eshghi et al. 2019). Epimutations are the cause of some rare diseases, such as Prader-Willi, Angelman or Beckwith-Wiedemann syndromes (Aref-Eshghi et al. 2019) and some human malformations (Serra-Juhé et al. 2015). Syndrome-specific episignatures are increasingly defined as biomarkers for a growing number of disorders (Aref-Eshghi et al. 2019; Garg et al. 2020). Therefore, tools to detect epimutations and episignatures should be made available to the rare disease community and included in standardized analysis workflows.

This manual describes the `epimutacions` package tools to identify epivariants using multiple outlier detection approaches. Also, includes functions to plot and annotate the epimutations.

The name of the package is `epimutacions` (pronounced `pi mu ta 'sj ons`) which means epimutations in Catalan, a language from the northeast of Spain.

1.2 Methodology

The `epimutacions` package computes a genome-wide DNA methylation analysis to detect the epigenetic variants to be considered as biomarkers for samples with rare diseases (epimutations). The method compares a case sample with suspected rare disease against a reference panel. The package focused on the detection of outliers in DNA methylation patterns associated with the diseases as proposed by (Aref-Eshghi et al. 2019).

The identification of relevant genomic methylation regions for a given sample having a rare disease will be driven by detecting differentially methylated CpG sites when comparing beta values of all control samples with the given proband. Firstly, bump-hunter (Jaffe et al. 2012) approach is used to identify the Differentially Methylated Regions (DMRs). After that, CpGs in the proband sample are tested in those DMRs in order to identify regions with CpGs being outliers when comparing with the reference panel. To this end, different anomaly detection statistical approaches are used. These include Multivariate Analysis of Variance (MANOVA) (Friedrich et al. 2017), Multivariate Linear Model (Martín 2020), isolation forest (Cortes and Cortes 2021) and robust mahalanobis distance (Maechler et al. 2021). However, Barbosa (M et al. 2018) and Beta methods do not use bump-hunter output. Barbosa (M et al. 2018) checks for each CpG, if the proband's measurement is an outlier. Then, it calls an epimutation to those regions where 3 contiguous CpGs are outliers, and they are separated by less than 500 base pairs. Beta approach models the DNA methylation data using a beta distribution.

1.3 Input data

The package allows two different types of inputs:

- (1) Case samples `IDAT` files (raw microarray intensities) together with `RGChannelSet` class object as reference panel. The reference panel can be supplied by the user or can be selected through the example datasets that the package provides.
- (2) `GenomicRatioSet` class object containing case and control samples.

The input data should contain information about β values of CpG sites, phenotype and feature data.

If you want to combine data from different studies, normalization is highly recommended. The preprocessing step removes the unwanted variation caused by the batch effect. In addition, it converts raw microarray intensities to `GenomicRatioSet` (necessary input class for `epimutations()` function). Finally, the case and control samples are introduced separately in `epimutations()` function.

The figure 1 shows the workflow related to the data types, normalization and inputs of `epimutations()` function.

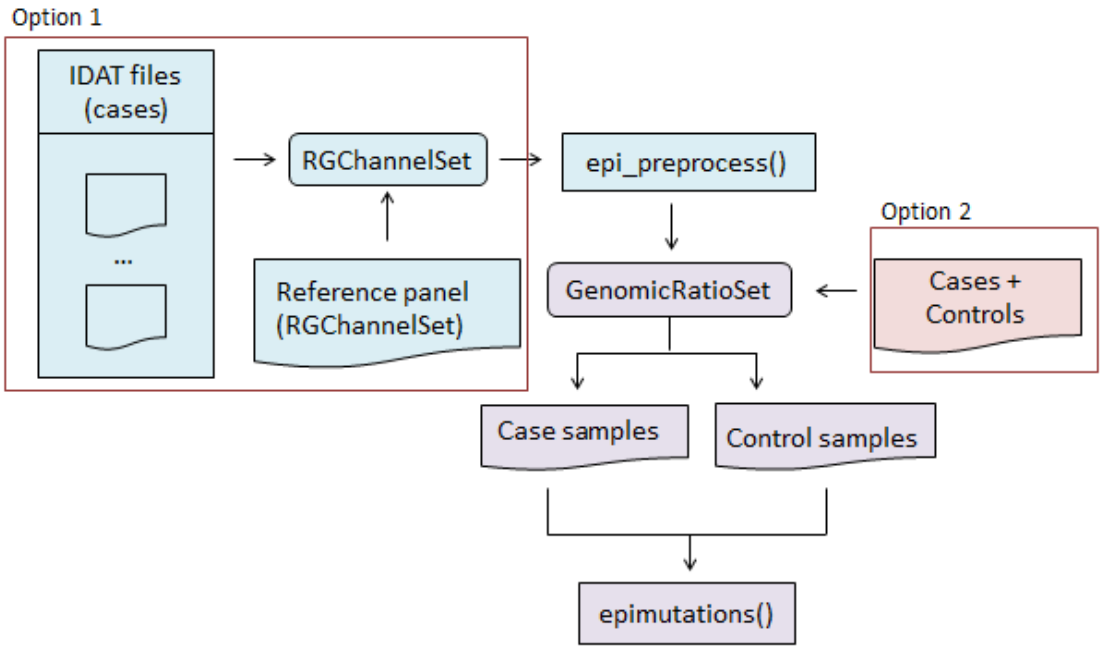


Figure 1: Allowed data formats, normalization and epimutations function input

2 Getting started

The `epimutacions` package is available on GitHub and can be installed by executing the following comand:

```
install_github("isglobal-brge/epimutacions")
```

The package is loaded in R as usual:

```
library(epimutacions)
```

The document has the following dependencies:

```
library(Knitr)
library(kableExtra)
```

3 Datasets

3.1 Candidate regions

Epimutations detection has two main steps: (1) definition of candidate regions and (2) evaluation of outlier significance. Although there are different algorithms to define epimutations regions, they share common features. In general, we define an epimutation as at least 3 contiguous CpGs with a maximum distance of 1kb between them (Aref-Eshghi et al. 2019; Garg et al. 2020).

In Illumina 450K array (Reproducibility 2012), probes are unequally distributed along the genome, limiting the number of regions that can fulfil the requirements to be considered an epimutation. So, we have computed a dataset containing the regions that are candidates to become an epimutation.

To define the candidate epimutations, we relied on the clustering from bumphunter (Jaffe et al. 2012). We defined a primary dataset with all the CpGs from the Illumina 450K array. Then, we run bumphunter and selected those regions with at least 3 CpGs. As a result, we found 40408 candidate epimutations which are available in `candRegsGR` dataset.

Besides, we converted the candidate region from hg19 to hg38 coordinates, using NCBI remap (Holmes et al. 2020). We selected regions that mapped to one region in hg38 with the same length. This yielded a total of 39944, the 98.85% of total hg19 regions. After converting to hg38, we can use these ranges to be annotated to ENCODE cREs (Hon and Carninci 2020). Overall, we mapped 30163 candidate regions to cREs, representing 74.65% of total candidate regions.

```
#Candidate regions dataset
##load data
data("candRegsGR")
##data class
class(candRegsGR)
```

```
[1] "GRanges"
attr(,"package")
[1] "GenomicRanges"
```

```
##dataset
candRegsGR
```

```
GRanges object with 40408 ranges and 9 metadata columns:
                 seqnames              ranges strand |     value      area
                    <Rle>           <IRanges>  <Rle> | <numeric> <numeric>
   chr6_32128101     chr6   32128101-32173532      * |         1       381
   chr6_33156164     chr6   33156164-33181870      * |         1       291
   chr6_32034322     chr6   32034322-32059605      * |         1       239
   chr6_31618987     chr6   31618987-31639143      * |         1       234
   chr6_33279563     chr6   33279563-33292029      * |         1       233
             ...      ...                 ...    ... .       ...       ...
  chr9_140652685     chr9 140652685-140652743      * |         1         3
  chr9_140656200     chr9 140656200-140657381      * |         1         3
  chr9_140680393     chr9 140680393-140681206      * |         1         3
  chr9_140732731     chr9 140732731-140733980      * |         1         3
  chr9_141012312     chr9 141012312-141013537      * |         1         3
                  cluster indexStart  indexEnd         L  clusterL
                <numeric>  <integer> <integer> <numeric> <integer>
   chr6_32128101    133070     165174    165554       381       381
```

```
  chr6_33156164    133204     167451    167741       291       291
  chr6_32034322    133058     164512    164750       239       239
  chr6_31618987    132987     162583    162816       234       234
  chr6_33279563    133221     168282    168514       233       233
            ...       ...        ...       ...       ...       ...
  chr9_140652685    162642     247198    247200         3         3
  chr9_140656200    162643     247201    247203         3         3
  chr9_140680393    162649     247214    247216         3         3
  chr9_140732731    162660     247252    247254         3         3
  chr9_141012312    162683     247294    247296         3         3
                                  CRE              CRE_type
                             <character>          <character>
  chr6_32128101 EH38E2459822,EH38E24.. pELS,CTCF-bound;PLS;..
  chr6_33156164 EH38E2460436,EH38E24.. PLS;pELS,CTCF-bound;..
  chr6_32034322 EH38E2459711,EH38E24.. dELS;dELS;dELS;pELS,..
  chr6_31618987 EH38E2459340,EH38E24.. pELS,CTCF-bound;PLS,..
  chr6_33279563 EH38E2460551,EH38E24.. pELS,CTCF-bound;pELS..
            ...                   ...                   ...
  chr9_140652685
  chr9_140656200 EH38E2738315,EH38E27.. pELS,CTCF-bound;pELS..
  chr9_140680393 EH38E2738332,EH38E27..   dELS,CTCF-bound;dELS
  chr9_140732731
  chr9_141012312
  -------
  seqinfo: 22 sequences from an unspecified genome; no seqlengths
```

3.2 GenomicRatioSet

The package includes a small `GenomicRatioSet` class dataset (`methy`) containing the DNA methylation profiles from a total of individuals, 3 cases and 48 controls. The DNA methylation profiles were generated using the Illumina 450k Human Methylation BeadChip (Reproducibility 2012). The data corresponds to GSE104812 cohort from Gene Expression Omnibus (GEO). It has been adapted for the package usage.

```
data("methy")
methy
```

```
class: GenomicRatioSet
dim: 80731 51
metadata(0):
assays(3): Beta M CN
rownames(80731): cg00725145 cg16080333 ... cg07468397 cg08821909
rowData names(0):
colnames(51): GSM2808239 GSM2808240 ... GSM2562700 GSM2562701
colData names(4): sampleID age sex status
Annotation
  array: IlluminaHumanMethylation450k
  annotation: ilmn12.hg19
Preprocessing
  Method: NA
  minfi version: NA
  Manifest version: NA
```

```
table(methy$status)
```

```
case control
   3      48
```

4 Preprocessing

The normalization removes the unwanted variation caused by the batch effect when combining data from different sources. The preprocessing in `epimutacions` package is done by `epi_preprocess()` function. It contains 6 preprocessing methods (table 1) corresponding to minfi package (Aryee et al. 2014), which can be selected by the user:

Table 1: Preprocessing methods description

Method	Function	Description
`raw`	`preprocessRaw`	Converts the Red/Green channel for an Illumina methylation array into methylation signal. This method does not normalize the data.
`illumina`	`preprocessIllumina`	Implements preprocessing for Illumina methylation microarrays as used in Genome Studio.
`swan`	`preprocessSWAN`	Subset-quantile Within Array Normalisation (SWAN). It allows Infinium I and II type probes on a single array to be normalized together.
`quantile`	`preprocessQuantile`	Implements stratified quantile normalization preprocessing for Illumina methylation microarrays.
`noob`	`preprocessNoob`	Noob (normal-exponential out-of-band) is a background correction method with dye-bias normalization for Illumina Infinium methylation arrays.
`funnorm`	`preprocessFunnorm`	Functional normalization (FunNorm) is a between-array normalization method for the Illumina Infinium HumanMethylation450 platform.

Those methods have unique parameters (table 3) that can be defined through `norm_parameters()` function:

Table 3: Preprocessing methods unique parameters

Method	Parameters	Description
`illumina`	`bg.correct`	Performs background correction
	`normalize`	Performs controls normalization
	`reference`	The reference array for control normalization
`quantile`	`fixOutliers`	Low outlier Meth and Unmeth signals will be fixed
	`removeBadSamples`	Remove bad samples
	`badSampleCutoff`	The cutoff to label samples as 'bad'
	`quantileNormalize`	Performs quantile normalization
	`stratified`	Performs quantile normalization within region strata
	`mergeManifest`	Merged to the output the information in the associated manifest package
	`sex`	Sex of the samples

Method	Parameters	Description
noob	offset	Offset for the normexp background correct
	dyeCorr	Performs dye normalization
	dyeMethod	Dye bias correction to be done
funnorm	nPCs	The number of principal components from the control probes
	sex	Sex of the samples
	bgCorr	Performs NOOB background correction prior to functional normalization
	dyeCorr	Performs dye normalization
	keepCN	Keeps copy number estimates

The default settings for each method can be obtained by invoking the function `norm_parameters()` with no arguments:

```
norm_parameters()
```

```
$illumina
$illumina$bg.correct
[1] TRUE

$illumina$normalize
[1] "controls" "no"

$illumina$reference
[1] 1


$quantile
$quantile$fixOutliers
[1] TRUE

$quantile$removeBadSamples
[1] FALSE

$quantile$badSampleCutoff
[1] 10.5

$quantile$quantileNormalize
[1] TRUE

$quantile$stratified
[1] TRUE

$quantile$mergeManifest
[1] FALSE

$quantile$sex
NULL


$noob
$noob$offset
[1] 15
```

```
$noob$dyeCorr
[1] TRUE

$noob$dyeMethod
[1] "single"    "reference"


$funnorm
$funnorm$nPCs
[1] 2

$funnorm$sex
NULL

$funnorm$bgCorr
[1] TRUE

$funnorm$dyeCorr
[1] TRUE

$funnorm$keepCN
[1] FALSE
```

However, to modify the parameters related to a method you can do as the following example for `illumina` approach:

```
parameters <- norm_parameters(illumina = list("bg.correct" = FALSE))
parameters$illumina$bg.correct
```

```
[1] FALSE
```

5 Epimutations

5.1 Epimutations detection

The `epimutacions` package includes 6 methods for epivariants identification: (1) Multivariate Analysis of variance (`manova`), (2) Multivariate Linear Model (`mlm`), (3) isolation forest (`isoforest`), (4) robust mahalanobis distance (`mahdistmcd`) (5) `barbosa` and (6) `beta`.
In the mentioned first 4 methods, firstly, Differentially Methylated Regions (DMRs) are identified using bump-hunter method (Jaffe et al. 2012). Then, those DMRs are tested to identify regions with CpGs being outliers when comparing with the reference panel. However, `barbosa` and `beta` do not identify outliers by filtering the DMRs. `barbosa` utilized a sliding window approach to individually compare the methylation value in each proband against the reference panel. `Beta` used beta distribution to identify epivariants in the case sample.
To ilustrate multiple examples we are going to use `methy` dataset. Fristly, we are going to split the dataset in two subset: (1) cases (`case_samples`) and (2) controls (`control_samples`):

```
case_samples <- methy[,methy$status == "case"]
control_samples <- methy[,methy$status == "control"]
```

Then, we are going to identify epimutations in each case sample using one statistical approach at a time:

```
epi_mvo <- epimutations(case_samples, control_samples, method = "manova")
epi_ml <- epimutations(case_samples, control_samples, method = "mlm")
epi_iso <- epimutations(case_samples, control_samples, method = "isoforest")
epi_mcd <- epimutations(case_samples, control_samples, method = "mahdistmcd")
epi_brb <- epimutations(case_samples, control_samples, method = "barbosa")
epi_beta <- epimutations(case_samples, control_samples, method = "beta")
```

5.2 Unique parameters

The `epi_parameters()` function is useful to set the individual parameters for each approach. The arguments are described in the table 5:

Table 5: epimutation function approaches unique parameters

Method	Parameter	Description
`manova`	`pvalue_cutoff`	The threshold p-value to select which CpG regions are outliers
`mlm`		
`beta`		
`iso.forest`	`outlier_score_cutoff`	The threshold to select which CpG regions are outliers
	`ntrees`	The number of binary trees to build for the model
`mahdist.mcd`	`nsamp`	The number of subsets used for initial estimates in the MCD
`barbosa`	`window_sz`	The maximum distance between CpGs to be considered in the same DMR
	`offset_mean/offset_abs`	The upper and lower threshold to consider a CpG an outlier
`beta`	`pvalue_cutoff`	The minimum p-value to consider a CpG an outlier
	`diff_threshold`	The minimum methylation difference between the CpG and the mean methylation to consider a position an outlier

Invoking `epi_parameters()` with no arguments returns a list of the default settings for each method:

```
epi_parameters()
```

```
$manova
$manova$pvalue_cutoff
[1] 0.05


$mlm
$mlm$pvalue_cutoff
[1] 0.05


$isoforest
$isoforest$outlier_score_cutoff
[1] 0.5

$isoforest$ntrees
[1] 100


$mahdistmcd
```

```
$mahdistmcd$nsamp
[1] "deterministic"


$barbosa
$barbosa$window_sz
[1] 10

$barbosa$offset_mean
[1] 0.15

$barbosa$offset_abs
[1] 0.1


$beta
$beta$pvalue_cutoff
[1] 1e-06

$beta$diff_threshold
[1] 0.1
```

The set up of any parameter can be done as the following example of p-value cut-off for `manova`:

```
parameters <- epi_parameters(manova = list("pvalue_cutoff" = 0.01))
parameters$manova$pvalue_cutoff
```

```
[1] 0.01
```

5.3 Results description

The `epimutations` function returns a `tibble` containing all the epivariants identified in the given case sample. In case no epimutation is found, a row containing the case sample information and missing values for each argument is returned. The table 7 describes each argument in the result data frame:

Table 7: epimutation function output arguments description

Column name	Description
`epi_id`	Systematic name for each epimutation identified
`sample`	The name of the sample containing that epimutation
`chromosome`	The location of the epimutation
`start end`	
`sz`	The window's size of the event
`cpg_n`	The number of CpGs in the epimutation
`cpg_n`	The names of CpGs in the epimutation
`outlier_score`	For method `manova` it provides the approximation to F-test and the Pillai score, separated by `/` For method `mlm` it provides the approximation to F-test and the R2 of the model, separated by `/` For method `isoforest` it provides the magnitude of the outlier score. For method `beta` it provides the mean p-value of all GpGs in that DMR For methods `barbosa` and `mahdistmcd` it is filled with `NA`.
`pvalue`	For methods `manova` and `mlm` it provides the p-value obtained from the model. For method `barbosa`, `isoforest`, `beta` and `mahdistmcd` it is filled with `NA`.

Column name	Description
`outlier_direction`	Indicates the direction of the outlier with "hypomethylation" and "hypermethylation". For `manova`, `mlm`, `isoforest`, and `mahdistmcd` it is computed from the values obtained from `bumphunter`. For `beta` is computed from the p value for each CpG using `diff_threshold` and `pvalue_threshold` arguments. For `barbosa` it is computed from the location of the sample in the reference distribution (left vs. right outlier).
`adj_pvalue`	For methods `manova` and `mlm` it provides the adjusted p-value with Benjamini-Hochberg based on the total number of regions detected by Bumphunter. For method `barbosa`, `isoforest`, `mahdistmcd` and `beta` it is filled with `NA`.
`epi_region_id`	Name of the epimutation region as defined in `candRegsGR`.
`CRE`	cREs (cis-Regulatory Elements) as defined by ENCODE overlapping the epimutation region.
`CRE_type`	Type of cREs (cis-Regulatory Elements) as defined by ENCODE.

5.4 Epimutations annotations

The `epimutacions` package also includes the `annotate_epimutations()` function dedicated to enriching the epimutations identified by the previously described methods:

```
rst_mvo <- annotate_epimutations(epi_mvo)
```

```
rst_mvo[1:2, c(1, 12:14)]
```

Table 9: epimutations annotation

	epi_id	adj_pvalue	epi_region_id	CRE
1	epi_manova_1	0.0000000	chr19_12776725	EH38E1939817,EH38E1939818,EH38E1939819
29	epi_manova_47	0.0116991	chr7_73894573	EH38E2563868,EH38E2563869

5.5 Epimutation visualization

The visualization approach locates the epimutations along the genome. The function `plot_epimutations()` plots the methylation values of the individual with the epimutation in red, the control samples in dashed black lines and population mean in blue:

```
plot_epimutations(as.data.frame(epi_mvo[1,]), methy)
```

Furthermore, it includes the gene annotations in the regions in which the epivariation is located. This can be achieved by using the argument `gene_annot == TRUE` (figure **??**):

```
plot_epimutations(as.data.frame(epi_mvo[1,]), methy, genes_annot = TRUE)
```

Also, it is possible to plot the chromatin marks H3K4me3, H3K27me3 and H3K27ac by setting the argument `regulation = TRUE`:

- **H3K4me3**: commonly associated with the activation of transcription of nearby genes.
- **H3K27me3**: is used in epigenetics to look for inactive genes.
- **H3K27ac**: is associated with the higher activation of transcription and therefore defined as an active enhancer mark

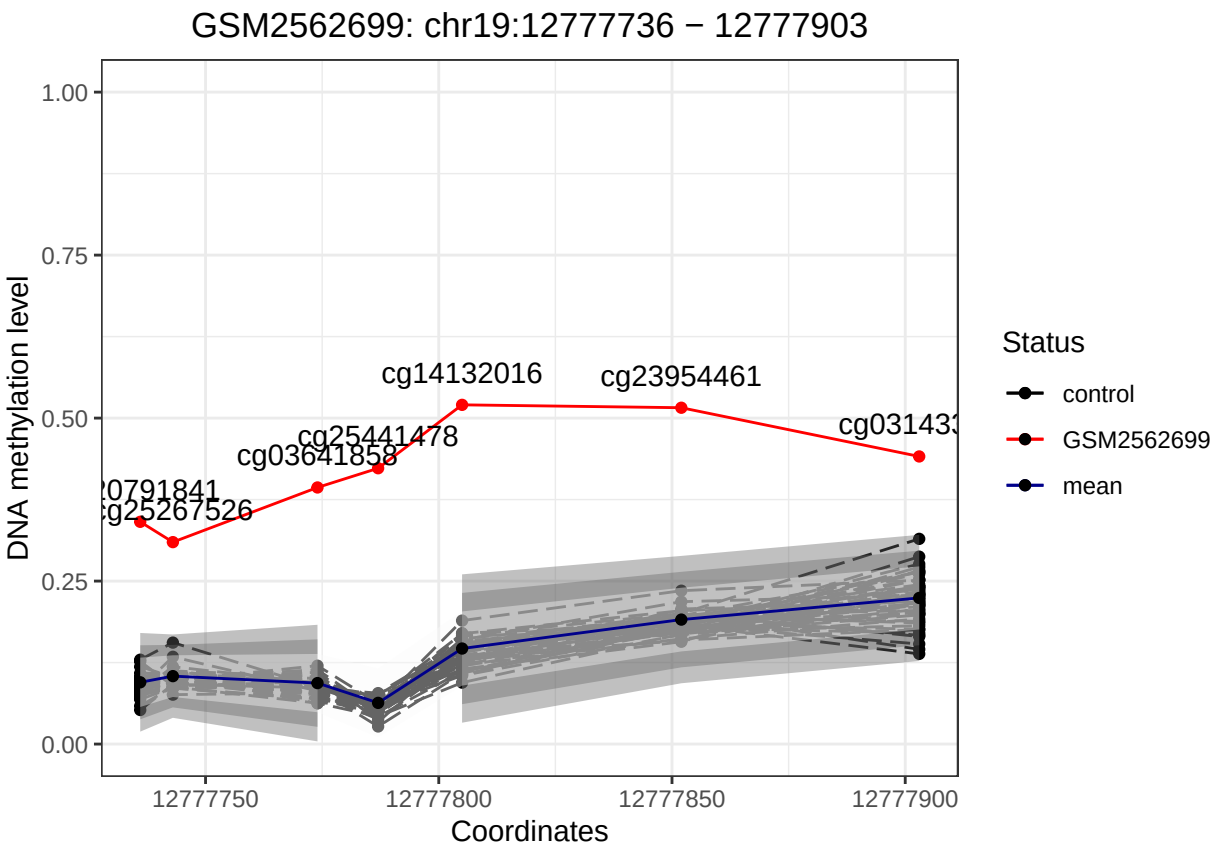


Figure 2: beta values of case sample againts control samples

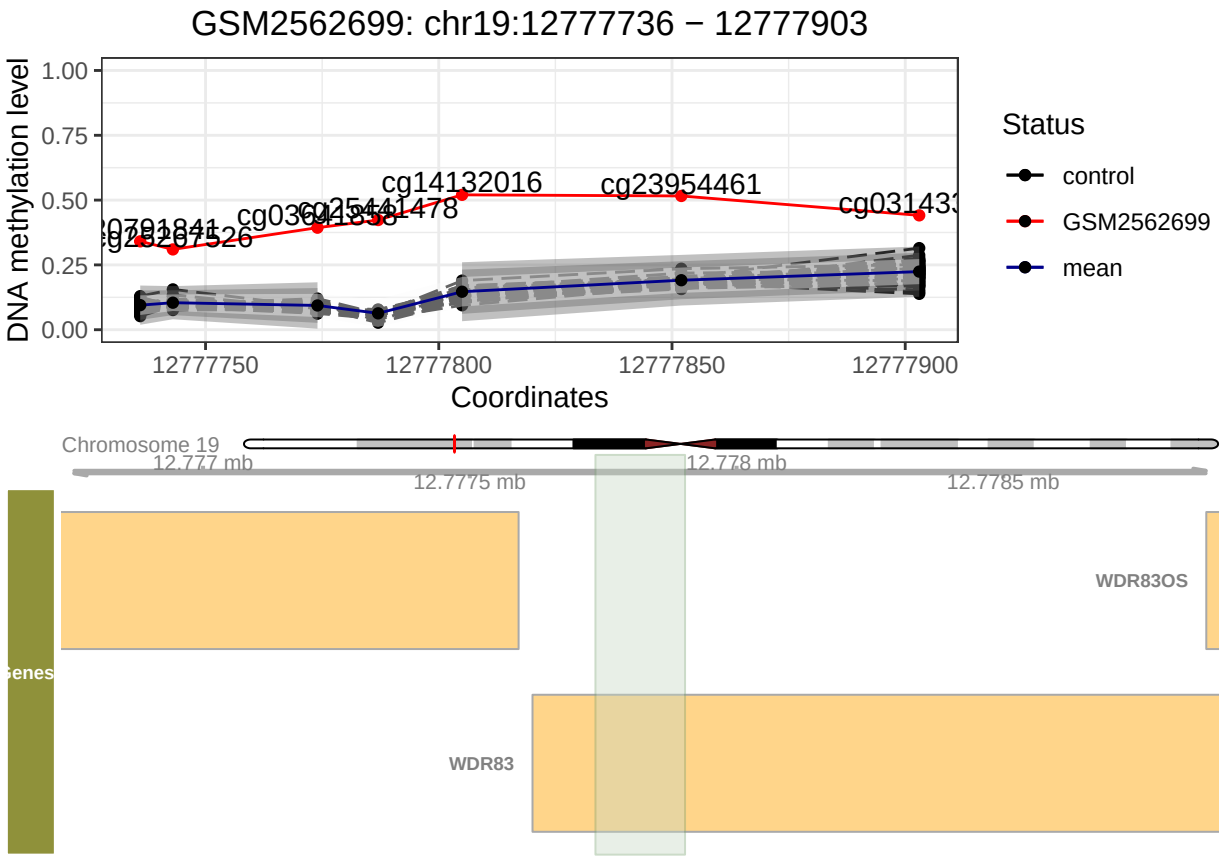
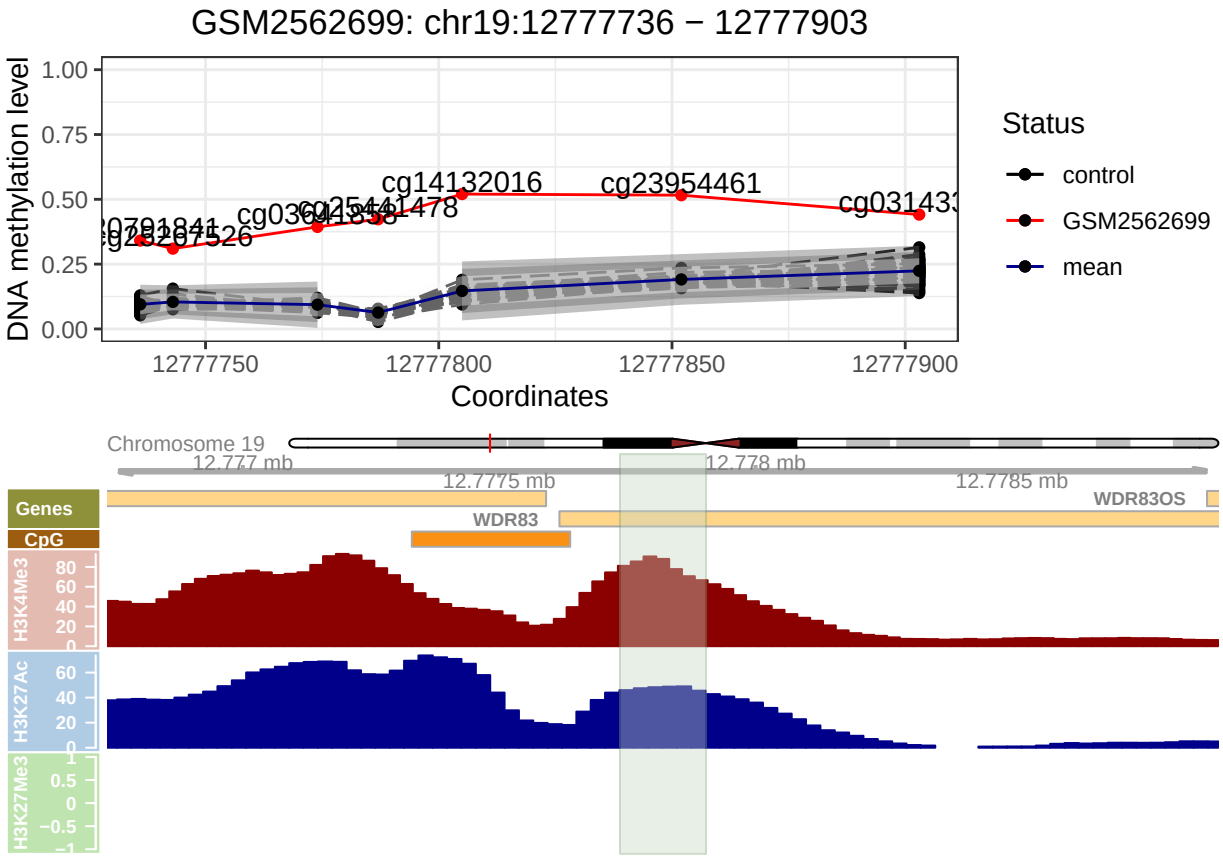


Figure 3: beta values of case sample againts control samples including UCSC gene annotation

```
plot_epimutations(as.data.frame(epi_mvo[1,]), methy, regulation = TRUE)
```



6 Method validation

We validate our method (`epimutacions`) reproducing the results in the study (Garg et al. 2020).

6.1 Data collection

The data were obtained from the study previously described (Garg et al. 2020). The datasets were downloaded from Gene Expression Omnibus (GEO). We accessed DNA methylation data from a total 1, 417 individuals from GSE51032 and GSE111629 cohorts. The DNA methylation profiles were generated using the Illumina 450k Human Methylation BeadChip.
The GSE51032 analysed primary cancers samples: 424 cancer free, 235 primary breast cancer, 166 primary colorectal cancer and 20 other primary cancers. The GSE111629 cohort 335 Parkinson's disease and 237 control samples.

6.2 Validation

We evaluated the performance of the method using True Positive Rate(TPR), False Positive Rate (FPR) and accuracy. We use the TPR to measure the proportion of detected epivariations by the `epimutations` approach present in the validated (table @ref(tab:tableEpiValidated)) by (Garg et al. 2020). FPR to calculate the identified epimutations outside the once found in (Garg et al. 2020), whether validated or not. The accuracy measures the closeness of the detected epimutation to the validated regions.
We select samples differently depending on the study group and measure to compute. Control samples were selected randomly using different sample size: 20, 30, 40, 50, 60, 70, 80, 90 and 100. However, case samples

were selected considering validated epimutations (for TPR and accuracy) or excluding epivariations found (for FPR) (Garg et al. 2020).
The validated epimutations in table 10 were only present on 5 individuals: GSM1235784 from GSE51032 cohort and GSM3035933, GSM3035791, GSM3035807 and GSM3035685 from GSE111629. Therefore, they were established as case samples when computing TPR and accuracy. Nevertheless, we compute FPR excluding the samples containing at least one epimutation found by (Garg et al. 2020). For the remaining case samples, 4 were selected randomly in each execution.
We execute 100 times the same process for each control sample size. We define for the analysis regions of $\approx$ 20 kb containing $\geq$ 3 GpGs.

Table 10: validated epimutations (Garg et al. 2020).

Chromosome	Start	End	Width	Strand	Samples
chr17	46018653	46019185	533	*	GSM1235784/GSM3035791
chr19	11199850	11200147	298	*	GSM3035685
chr5	10249760	10251253	1494	*	GSM3035933
chr5	67583971	67584381	411	*	GSM3035791/GSM3035807

Additionally, we have plotted the methylation values of the samples in the regions where the validated epimutations were found.

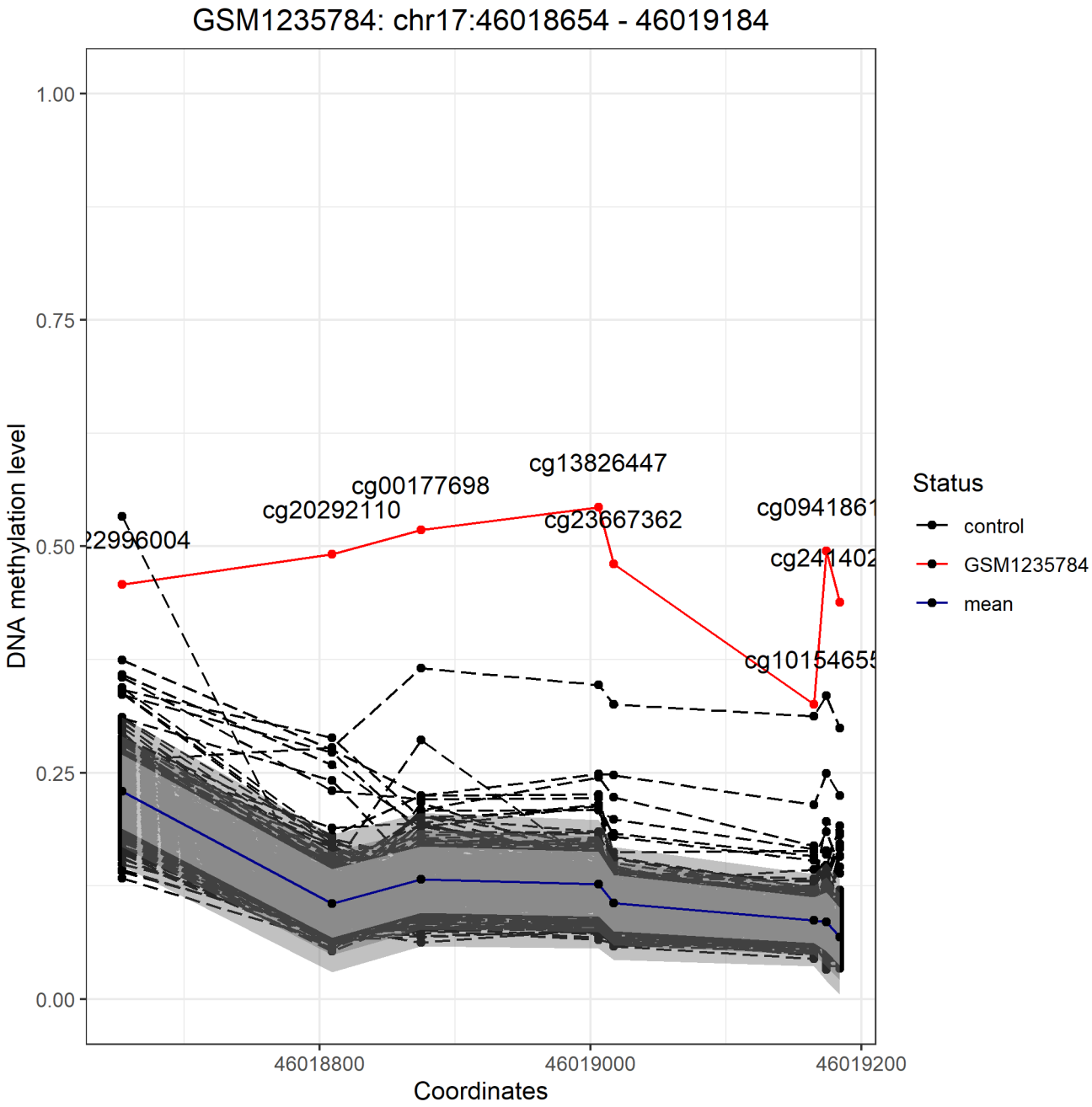


Figure 4: GSE51032 cohort samples in the region chr17:46018654-46019184

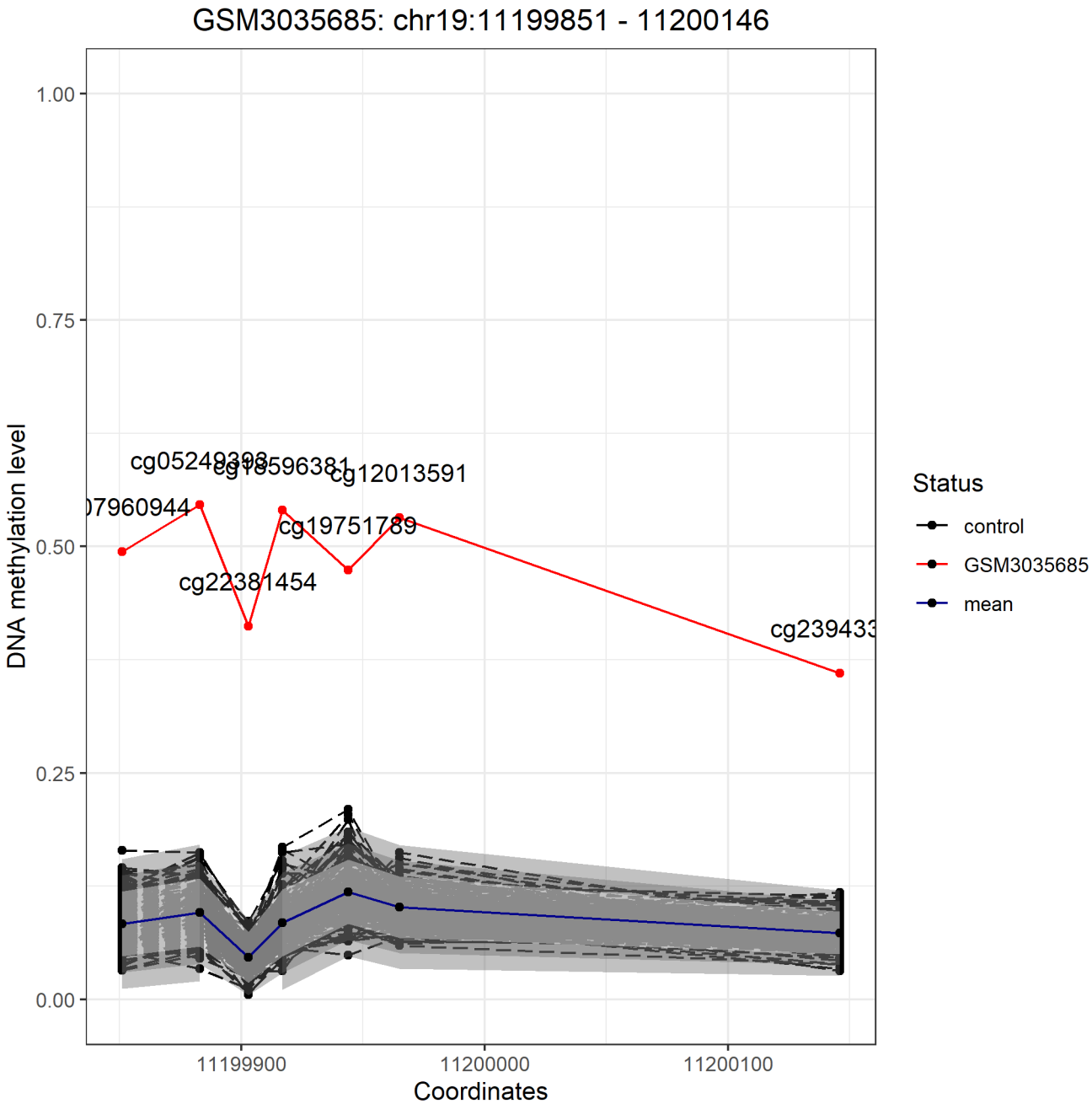


Figure 5: GSE111629 cohort samples in the region chr19:11199851-11200146

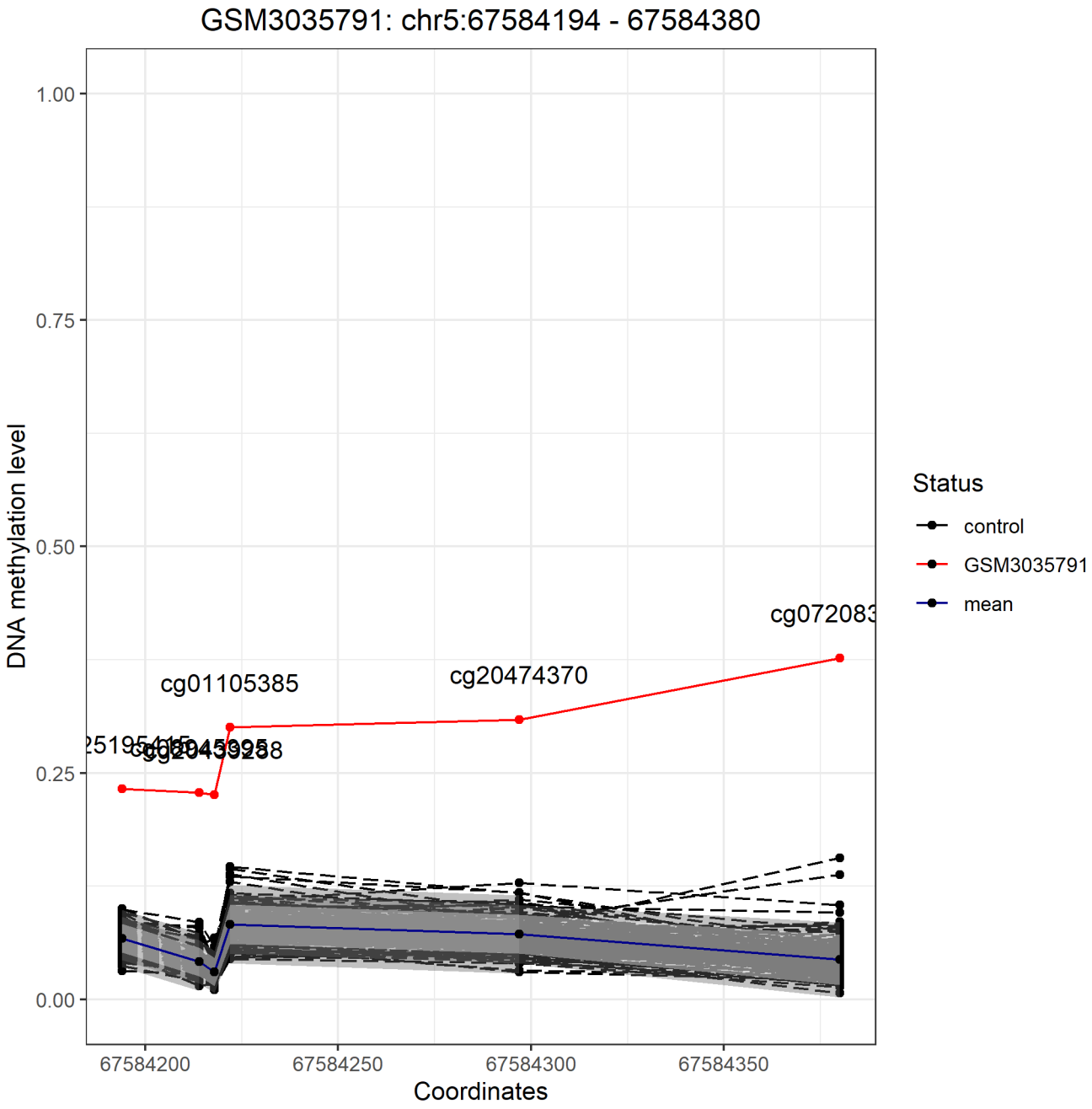


Figure 6: GSE111629 cohort samples in the region chr5:67584194-67584380

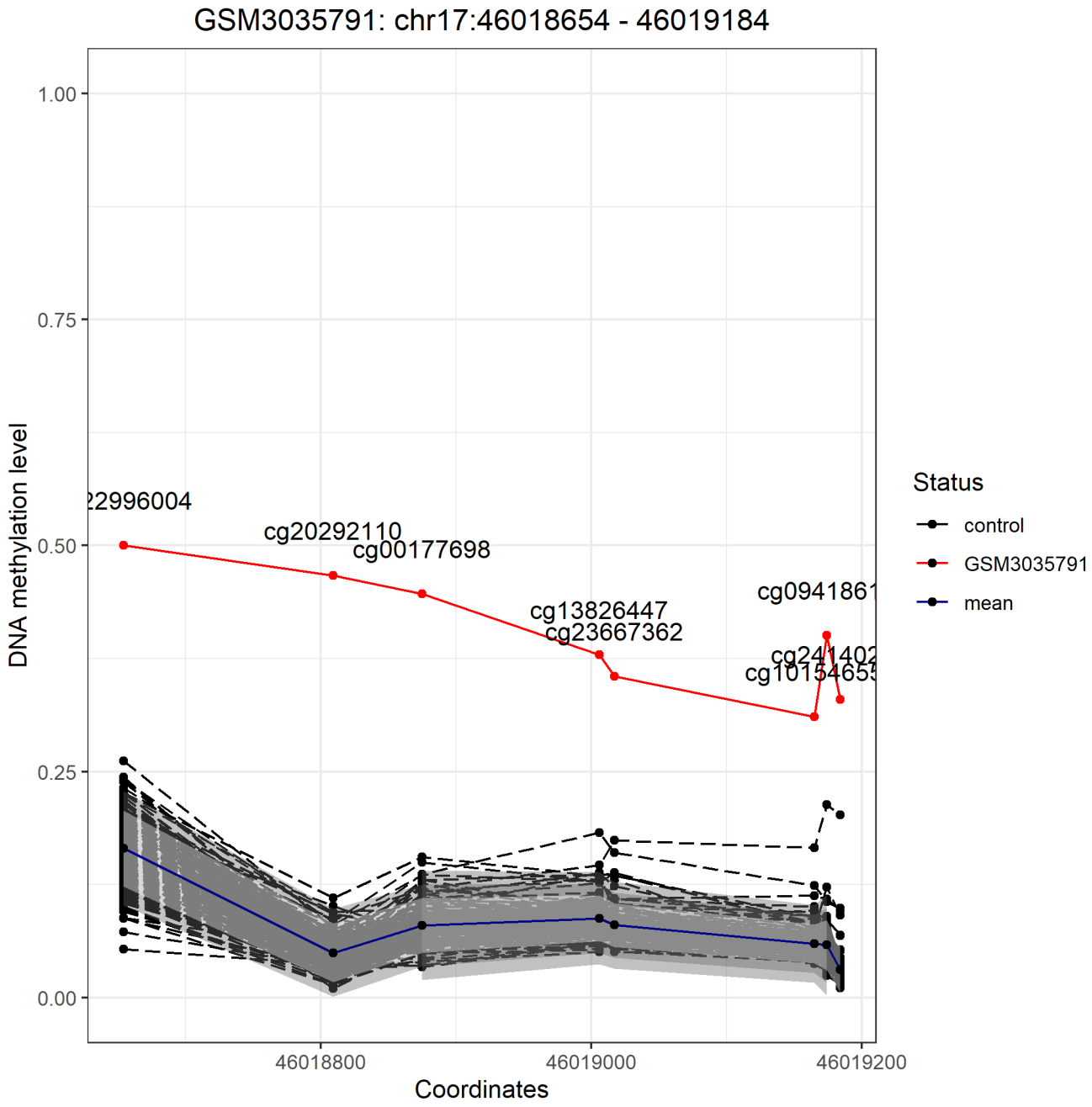


Figure 7: GSE111629 cohort samples in the region chr17:46018654-46019184

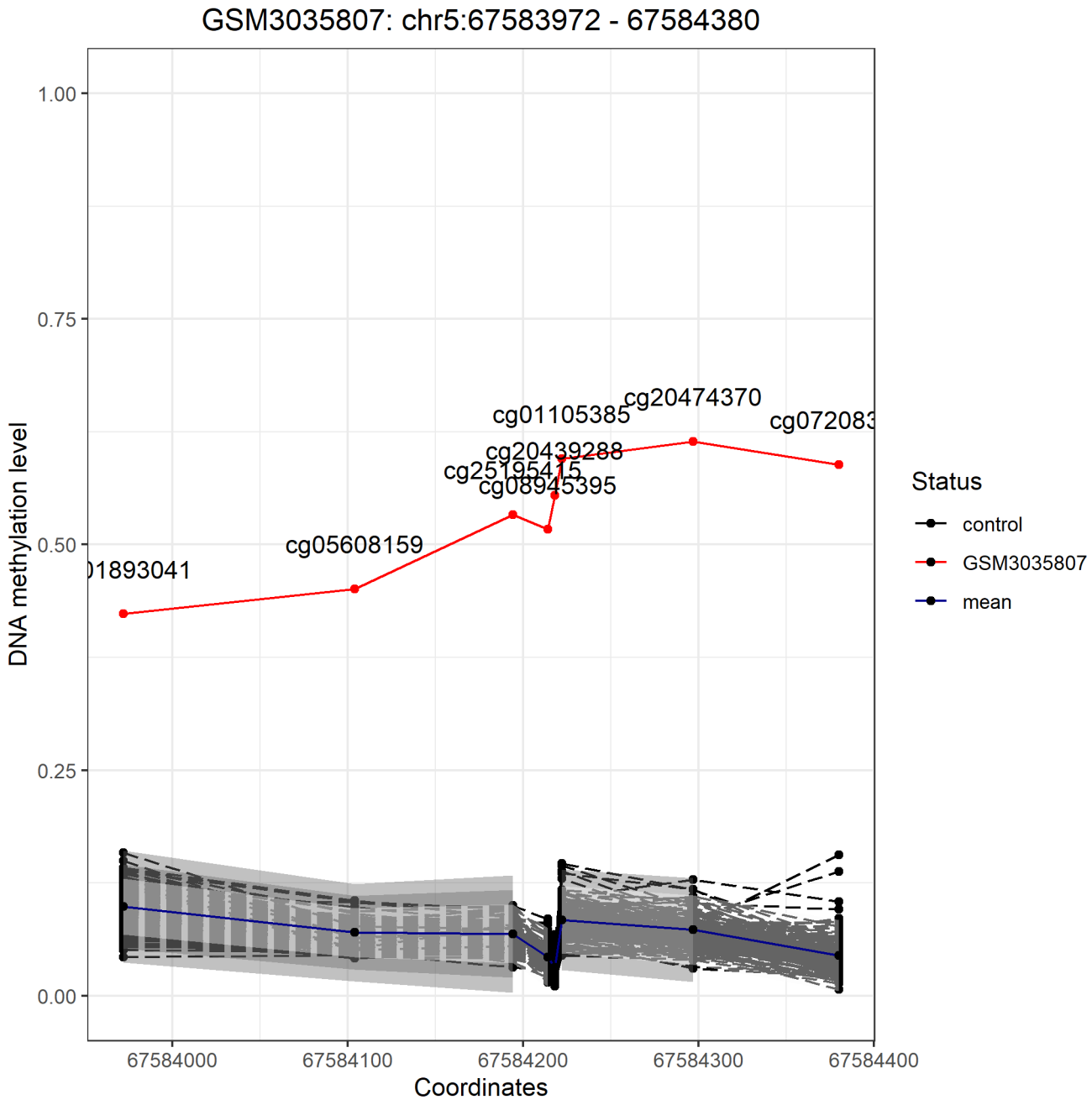


Figure 8: GSE111629 cohort samples in the region chr5:67583972-67584380

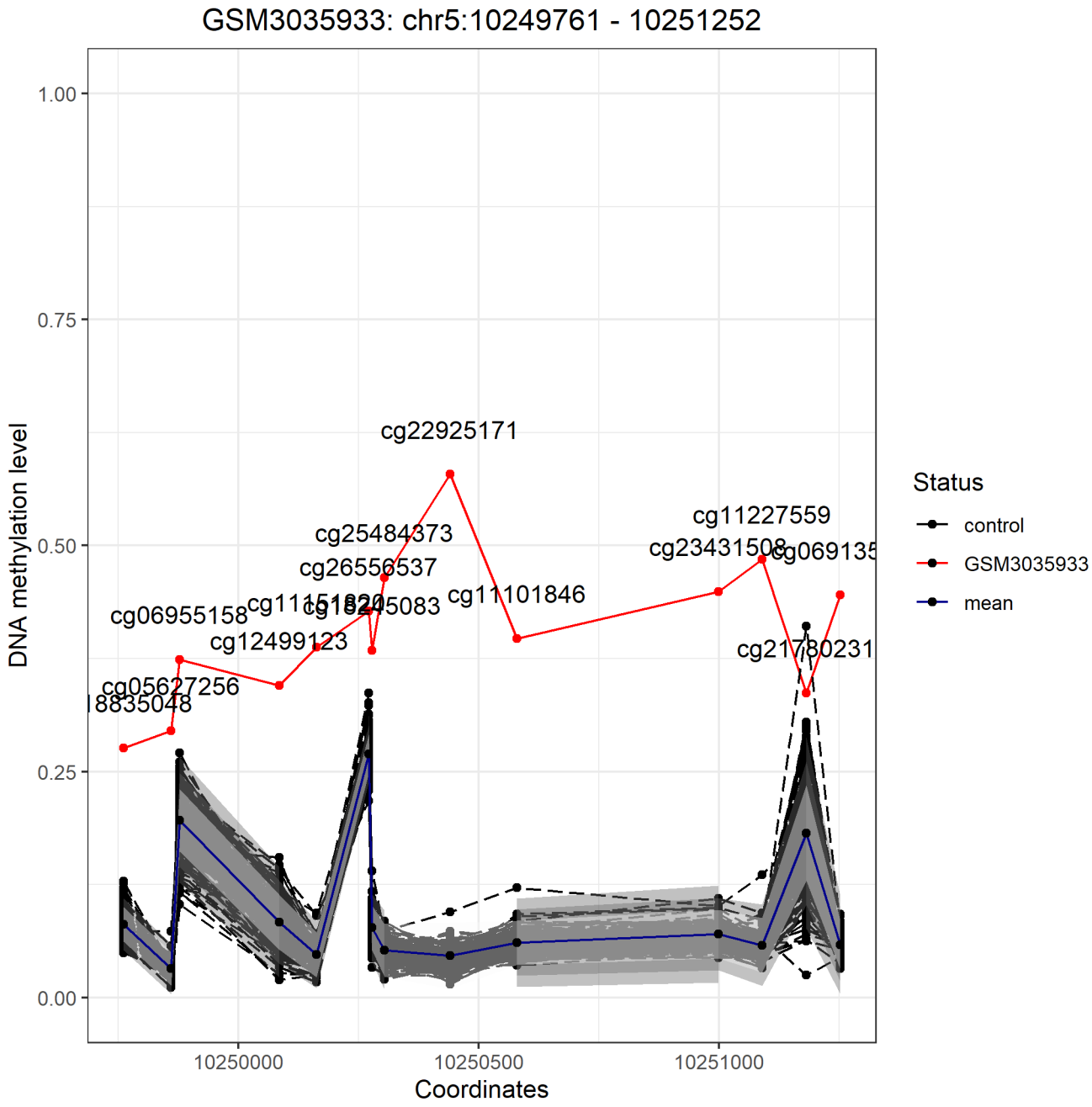


Figure 9: GSE111629 cohort samples in the region chr5:10249761-10251252

6.3 Results

We compared GSM1235784 case sample against randomly selected control samples from GSE51032. furthermore, GSM3035933, GSM3035791, GSM3035807 and GSM3035685 case samples were studied against controls from GSE111629 specifying a region of 20 kb and ≥ 3 GpGs.
We obtained similar results in both cohorts. We observed that the methods manova, mahalanobis distance, multivariate linear models, barbosa and beta identified the validated epimutations with a TPR of $> 99\%$ even if the control sample is small. However, the TPR in isolation forest increases together with the number of control samples obtaining a TPR ≥ 75 with 50 control samples or more. Regarding the accuracy, all the

statistical approaches detect the epivariants with $> 80\%$ of closeness to the validated epimutations.
We detected possible epivariations outside the epimutations found by (Garg et al. 2020) selecting random regions of 20 kb and ≥ 3 GpGs for both, control and case samples. We compared individual methylation profiles of a single case sample against control samples. We observed that in both cohorts and for every approach the FPR value is minimum $< 0.01\%$.

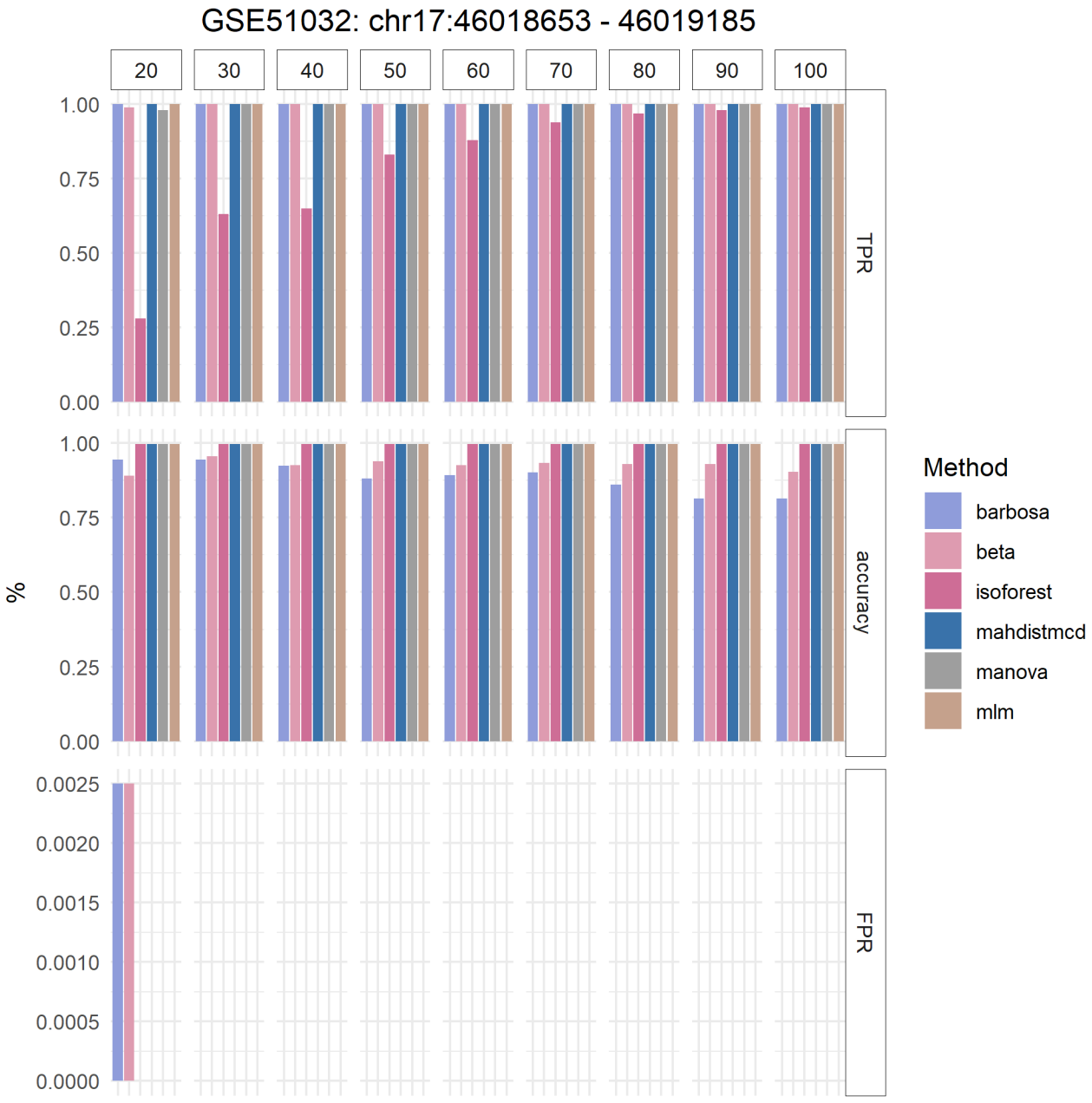


Figure 10: epimutations performance for GSE51032 cohort detecting the epivariation located in chr5:10249760-10251253

method	TPR	accuracy	FPR
n20			
manova	98	99.6	0.00
mlm	100	99.6	0.00
mahdistmcd	100	99.6	0.00
isoforest	28	99.6	0.00
barbosa	100	94.4	0.25
beta	99	89.0	0.25
n30			
manova	100	99.6	0.00
mlm	100	99.6	0.00
mahdistmcd	100	99.6	0.00
isoforest	63	99.6	0.00
barbosa	100	94.4	0.00
beta	100	95.4	0.00
n40			
manova	100	99.6	0.00
mlm	100	99.6	0.00
mahdistmcd	100	99.6	0.00
isoforest	65	99.6	0.00
barbosa	100	92.4	0.00
beta	100	92.6	0.00
n50			
manova	100	99.6	0.00
mlm	100	99.6	0.00
mahdistmcd	100	99.6	0.00
isoforest	83	99.6	0.00
barbosa	100	88.0	0.00
beta	100	93.8	0.00
n60			
manova	100	99.6	0.00
mlm	100	99.6	0.00
mahdistmcd	100	99.6	0.00
isoforest	88	99.6	0.00
barbosa	100	89.2	0.00
beta	100	92.6	0.00
n70			
manova	100	99.6	0.00
mlm	100	99.6	0.00
mahdistmcd	100	99.6	0.00
isoforest	94	99.6	0.00
barbosa	100	90.0	0.00
beta	100	93.2	0.00
n80			
manova	100	99.6	0.00
mlm	100	99.6	0.00
mahdistmcd	100	99.6	0.00
isoforest	97	99.6	0.00
barbosa	100	86.0	0.00
beta	100	92.9	0.00
n90			
manova	100	99.6	0.00
mlm	100	99.6	0.00
mahdistmcd	100	99.6	0.00
isoforest	98	99.6	0.00
barbosa	100	81.3	0.00
beta	100	92.9	0.00
n100			

method	n	TPR	accuracy	FPR
n20				
barbosa	100	100.000	92.825	0.00
beta	100	100.000	92.825	0.25
isoforest	100	98.625	93.000	0.00
mahdistmcd	100	100.000	92.825	0.00
manova	100	100.000	92.825	0.00
mlm	100	100.000	92.825	0.00
n30				
barbosa	20	100.000	92.825	0.00
beta	20	87.500	92.800	0.00
isoforest	20	11.500	86.800	0.00
mahdistmcd	20	100.000	92.825	0.00
manova	20	98.000	92.825	0.00
mlm	20	100.000	92.850	0.00
n40				
barbosa	30	100.000	92.825	0.25
beta	30	87.500	92.775	0.25
isoforest	30	28.000	93.150	0.00
mahdistmcd	30	100.000	92.825	0.00
manova	30	100.000	92.825	0.00
mlm	30	100.000	92.825	0.00
n50				
barbosa	40	100.000	92.825	0.00
beta	40	87.500	92.800	0.00
isoforest	40	46.375	93.950	0.00
mahdistmcd	40	100.000	92.825	0.00
manova	40	100.000	92.825	0.00
mlm	40	100.000	92.825	0.00
n60				
barbosa	50	100.000	92.825	0.00
beta	50	87.500	92.825	0.00
isoforest	50	70.125	93.500	0.00
mahdistmcd	50	100.000	92.825	0.00
manova	50	100.000	92.825	0.00
mlm	50	100.000	92.825	0.00
n70				
barbosa	60	100.000	92.825	0.00
beta	60	100.000	92.825	0.00
isoforest	60	78.750	93.500	0.00
mahdistmcd	60	100.000	92.825	0.00
manova	60	100.000	92.825	0.00
mlm	60	100.000	92.825	0.00
n80				
barbosa	70	100.000	92.825	0.25
beta	70	100.000	92.825	0.00
isoforest	70	90.125	93.575	0.00
mahdistmcd	70	100.000	92.825	0.00
manova	70	100.000	92.825	0.00
mlm	70	100.000	92.825	0.00
n90				
barbosa	80	100.000	92.825	0.00
beta	80	100.000	92.825	0.00
isoforest	80	96.375	93.075	0.00
mahdistmcd	80	100.000	92.825	0.00
manova	80	100.000	92.825	0.00
mlm	80	100.000	92.825	0.00
n100				

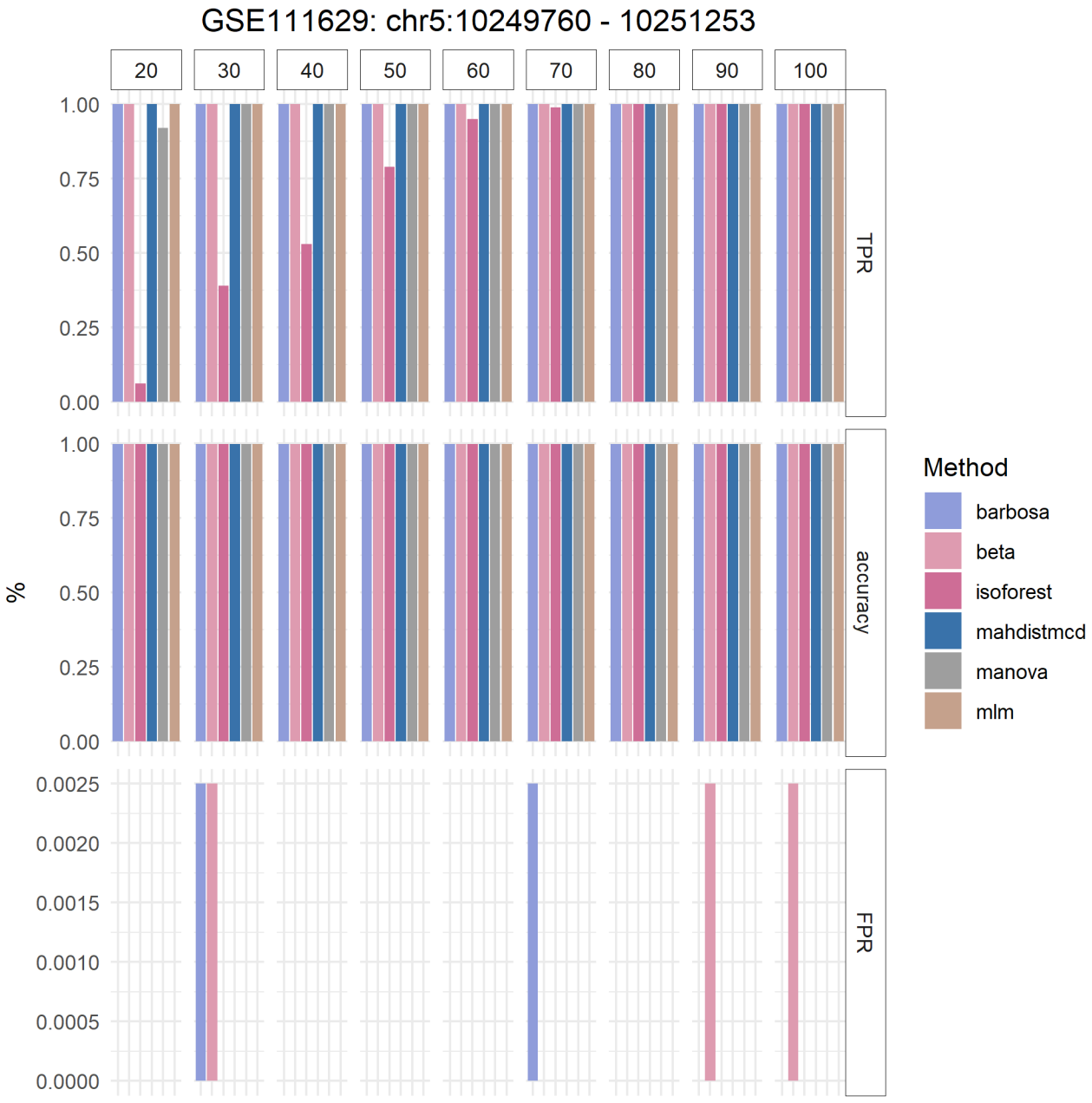


Figure 11: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:10249760-10251253

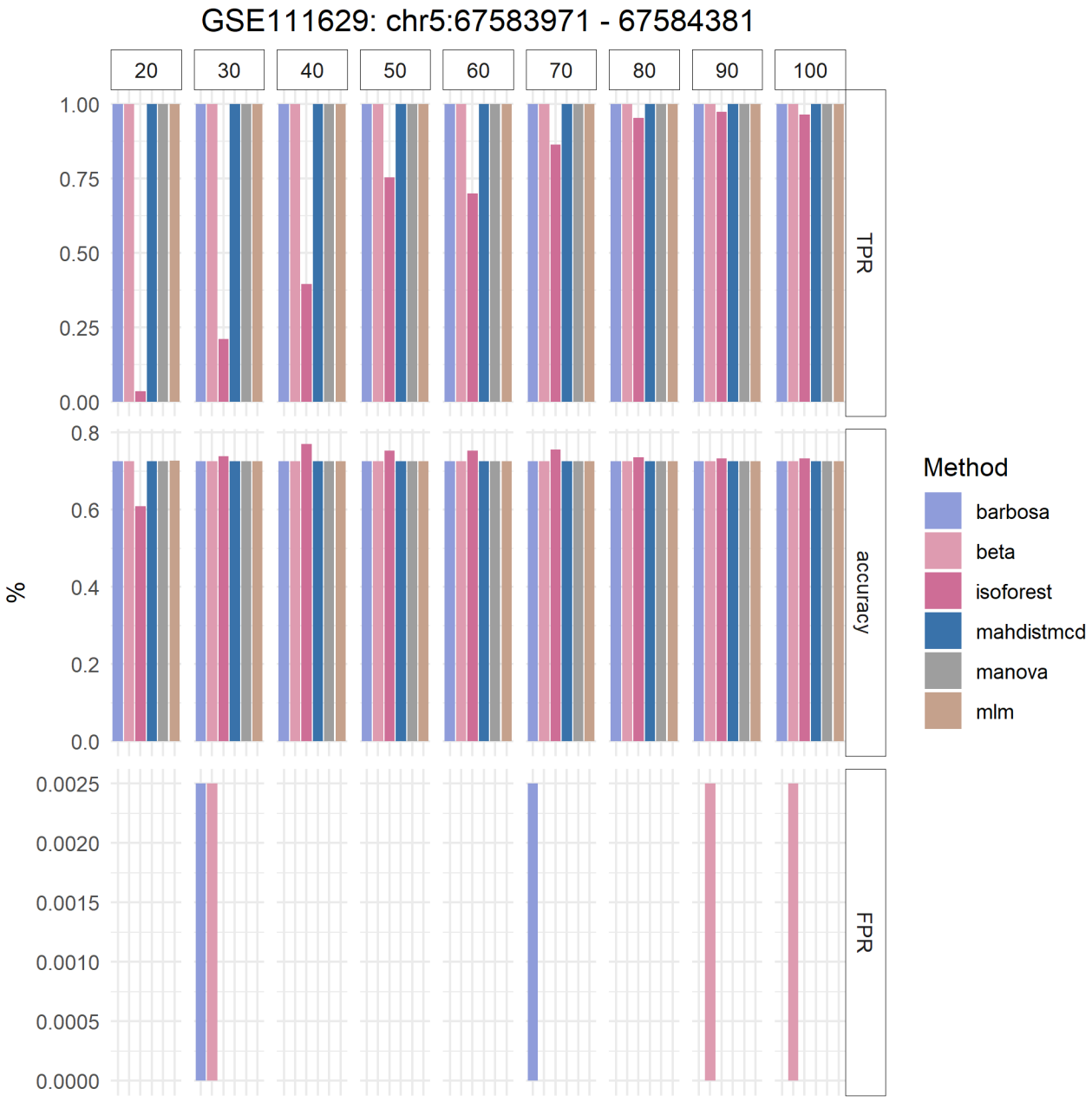


Figure 12: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:67583971-67584381

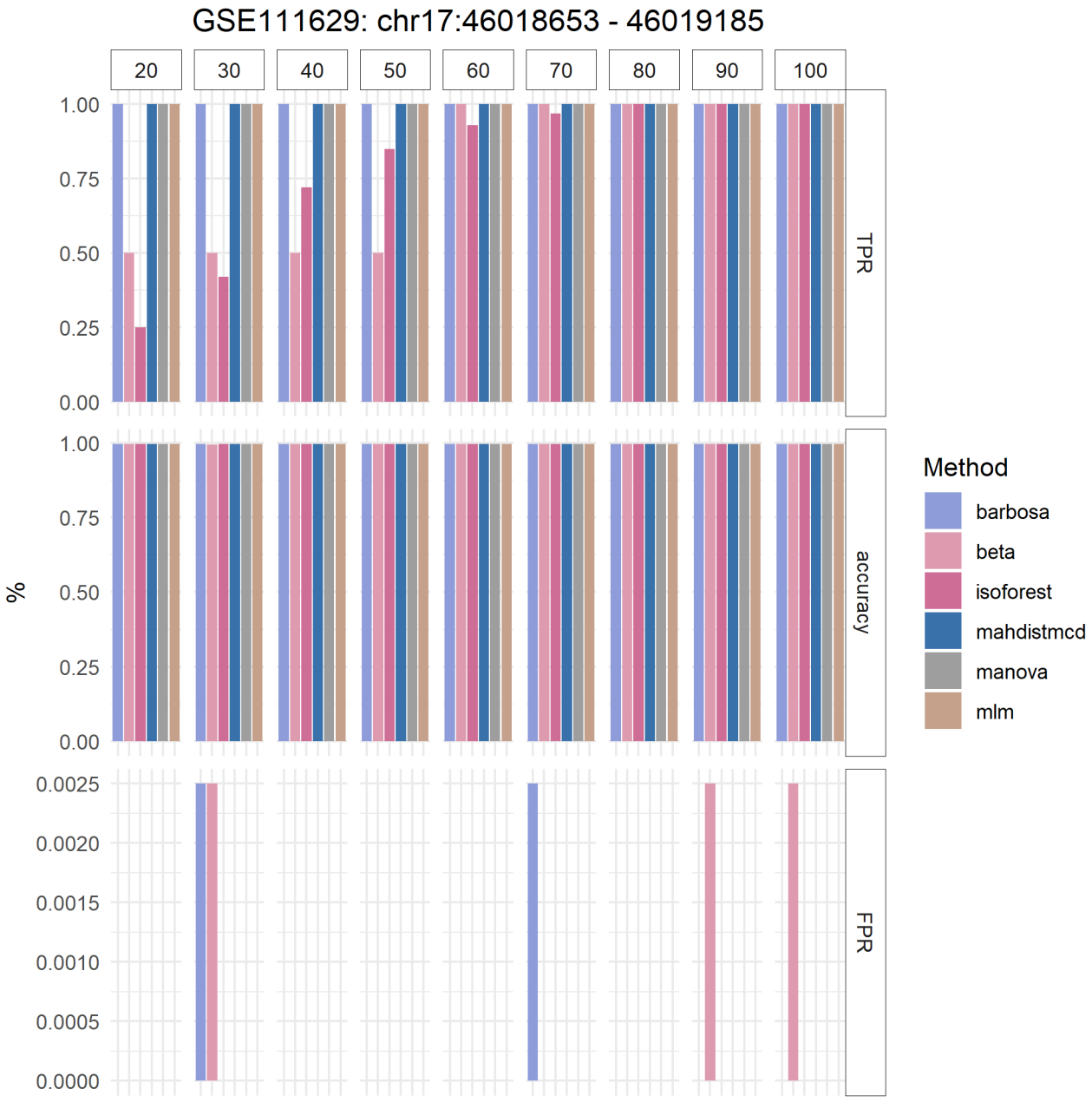


Figure 13: epimutations performance using GSE111629 cohort to detect the epivariation located in chr17:46018653-46019185

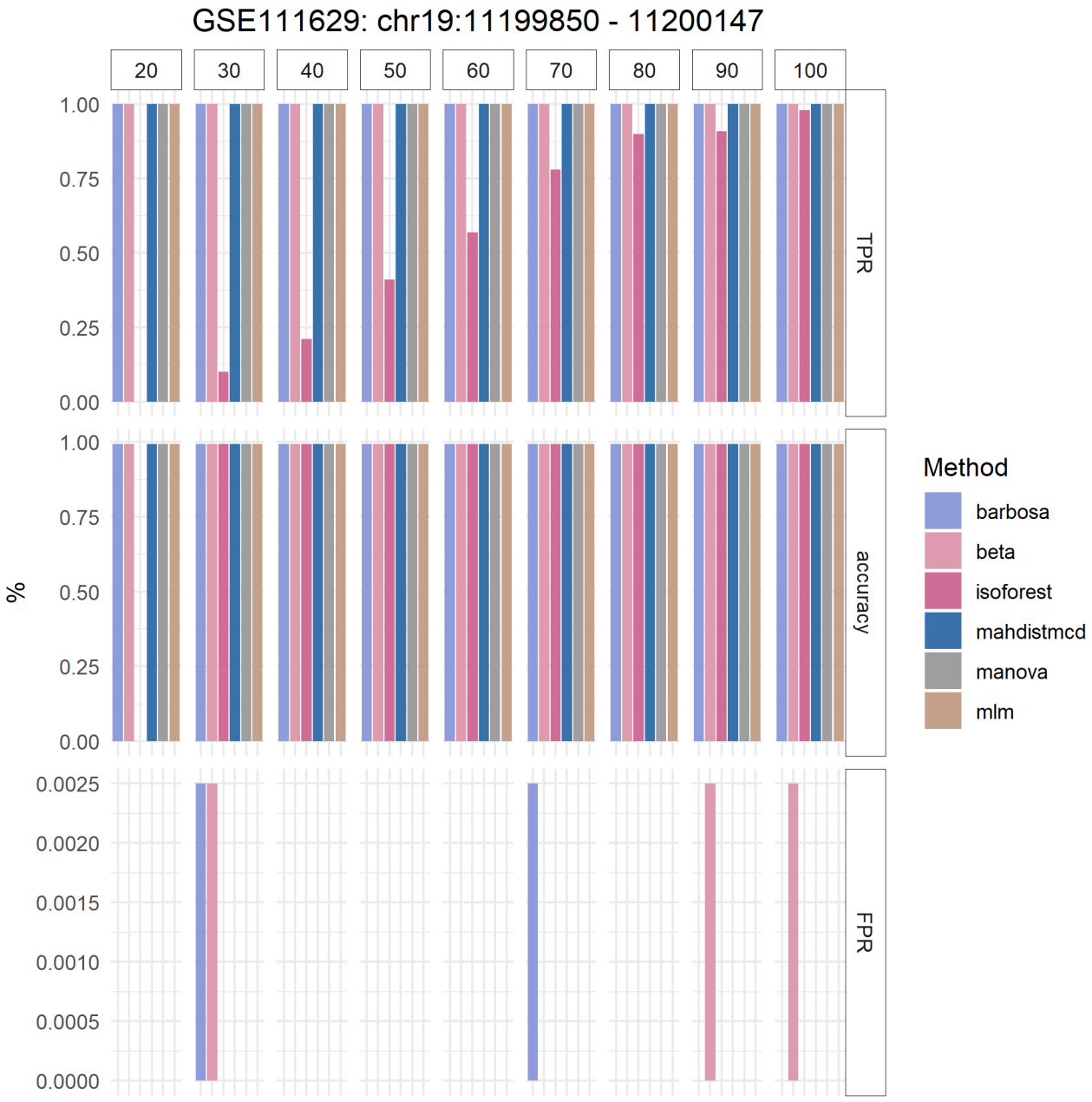


Figure 14: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:11199850-11200147

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All the team members of *Project #5* for the contribution to this package:

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