epimutacion package validation

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Introduction

Rare diseases are pathologies with a low prevalence (< 1 per 2,000 people). Most of these pathologies have an onset during childhood and a strong genetic etiology. Due to their low prevalence, there is a lack of knowledge which causes a delay in diagnosis and a lack of effective treatment. Thus, affecting the life expectancy and quality of the patient. Current methodologies for identifying mutations related to rare diseases have relied on Whole Genomic Sequencing. Despite exhaustive assessments, in a large proportion of case subjects, the underlying genetic etiology is not identified or the clinical assessment does not indicate a diagnosis. In order to overcome this difficulty, genome-wide DNA methylation analysis has been proposed to facilitate the molecular diagnosis of unresolved clinical cases and be consider for routine clinical assessment. We developed `epimutacions`, a method that detects outliers in DNA methylation patterns associated with the diseases as proposed by (Aref-Eshghi et al. 2019). We validated our method by performing simulations based on the data and results obtained in the survey (Garg et al. 2020).

Data collection

The data were obtained for the studies 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 study 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.

Validation

We evaluated the performance of the method using TPR (True Positive Rate), 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 (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 on table 1 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 1: 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

Results

We compare GSM1235784 case sample against randomly selected control samples from GSE51032 and GSM3035933, GSM3035791, GSM3035807 and GSM3035685 case samples against controls from GSE111629 specifying a region of 20 kb and $\geq$ 3 GpGs.

We obtained similar results in both cohorts. We observed that the methods manova, mahalanobis distance and multivariate linear models 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 $\geq$ 75 with 50 control samples or more. The TPR in barbosa and beta approaches for GSE51032 dataset is small ($< 50\%$). Nonetheless, for GSE111629 the TPR value increases considerably $> 99\%$. Regarding to 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 control and case samples randomly. For the analysis, we selected regions of 20 kb and $\geq$ 3 GpGs. We compared each case sample individually against control samples. We observed that in both cohorts and for every approach the FPR value is very small $< 0.01\%$.

Table 2: `epimutations` function TPR, FPR and accuracy using GSE51032 cohort.

method	TPR	accuracy	FPR
barbosa	0.5000000	0.8855556	0.0002778
beta	0.4994444	0.9252222	0.0002778
isoforest	0.7944444	0.9960000	0.0000000
mahdistmcd	1.0000000	0.9960000	0.0000000
manova	0.9977778	0.9960000	0.0000000
mlm	1.0000000	0.9960000	0.0000000

Figure 1: epimutations performance for GSE51032 cohort detecting the epivariation located in chr5:10249760-10251253

Table 3: `epimutations` function TPR, FPR and accuracy for GSE111629 cohort. The measures shown are the mean for all the validated epimutations identified.

method	TPR	accuracy	FPR
barbosa	1.0000000	0.9282500	0.0005556
beta	0.9444444	0.9281389	0.0008333
isoforest	0.7018571	0.9278286	0.0000000
mahdistmcd	1.0000000	0.9282500	0.0000000

method	TPR	accuracy	FPR
manova	0.9977778	0.9282500	0.0000000
mlm	1.0000000	0.9282778	0.0000000

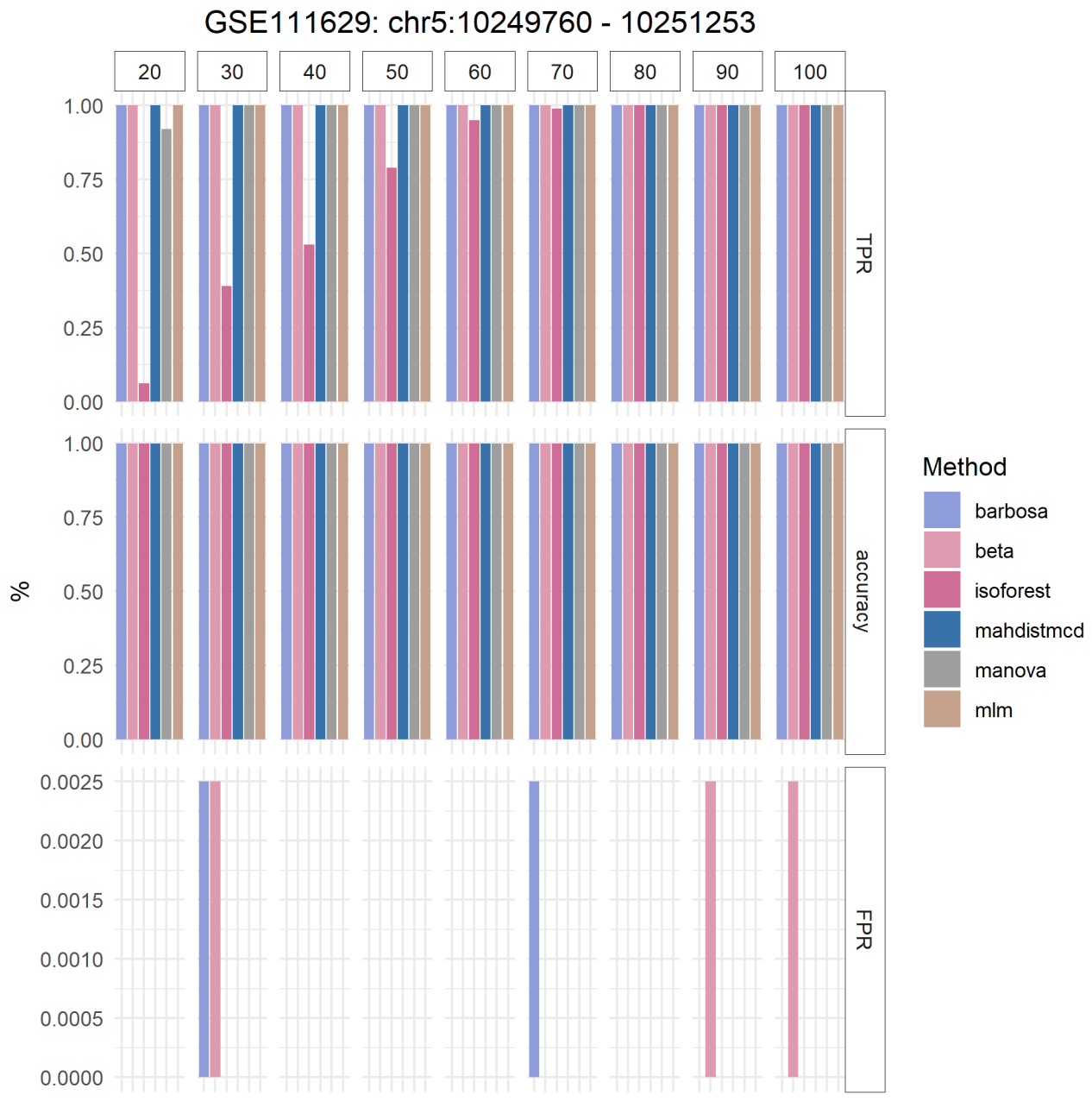

Figure 2: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:10249760-10251253

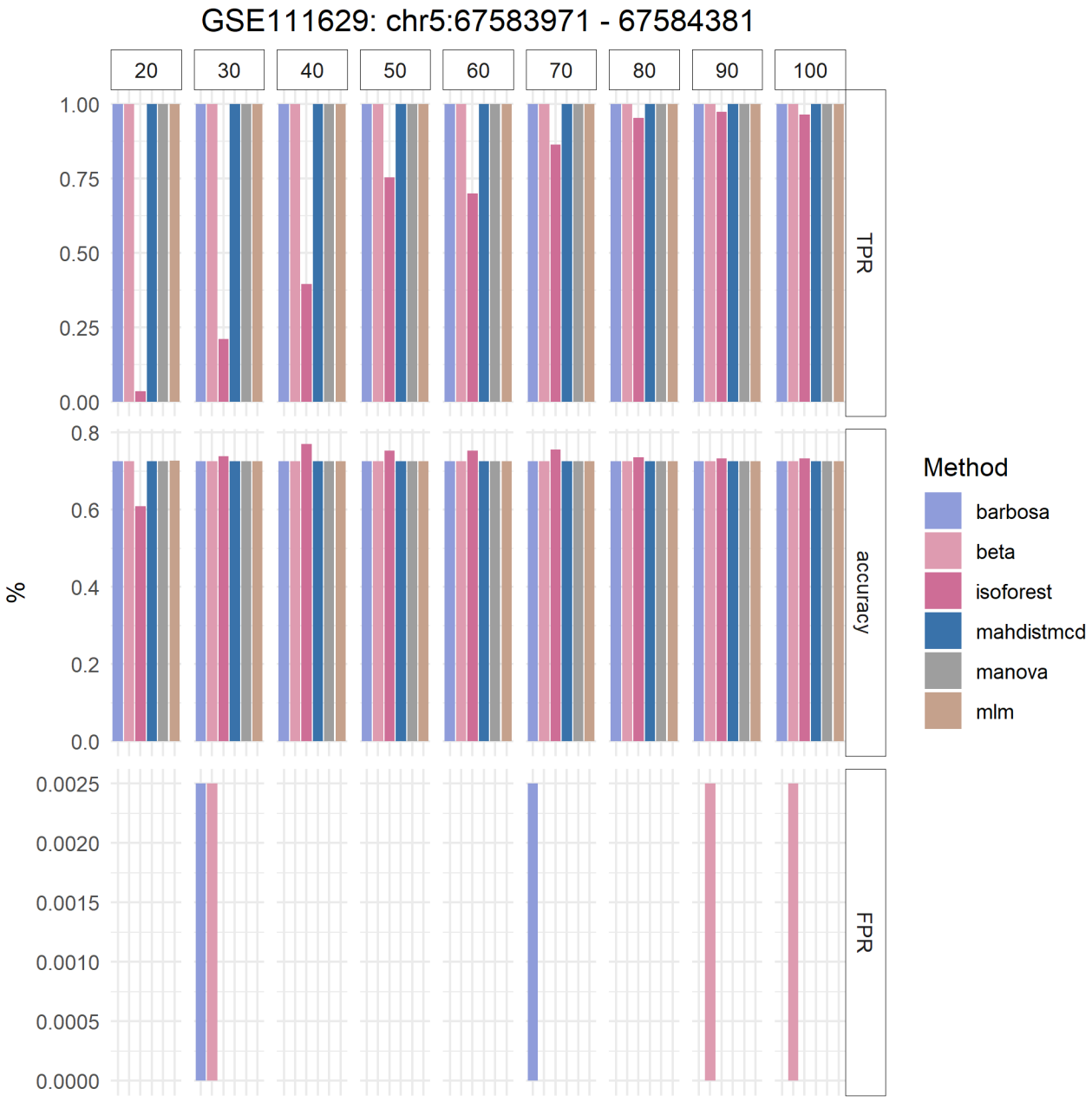

Figure 3: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:67583971-67584381

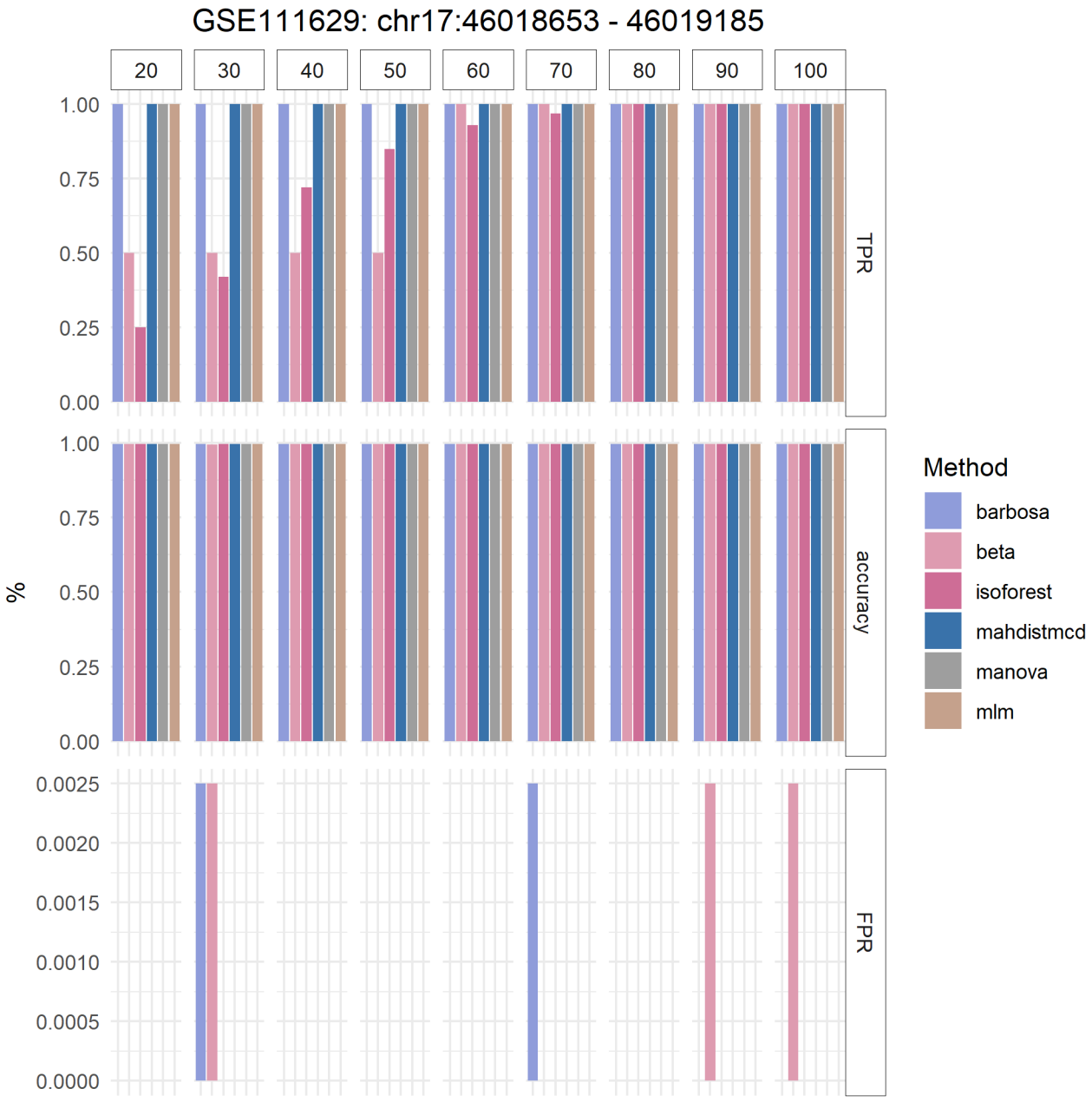

Figure 4: epimutations performance using GSE111629 cohort to detect the epivariation located in chr17:46018653-46019185

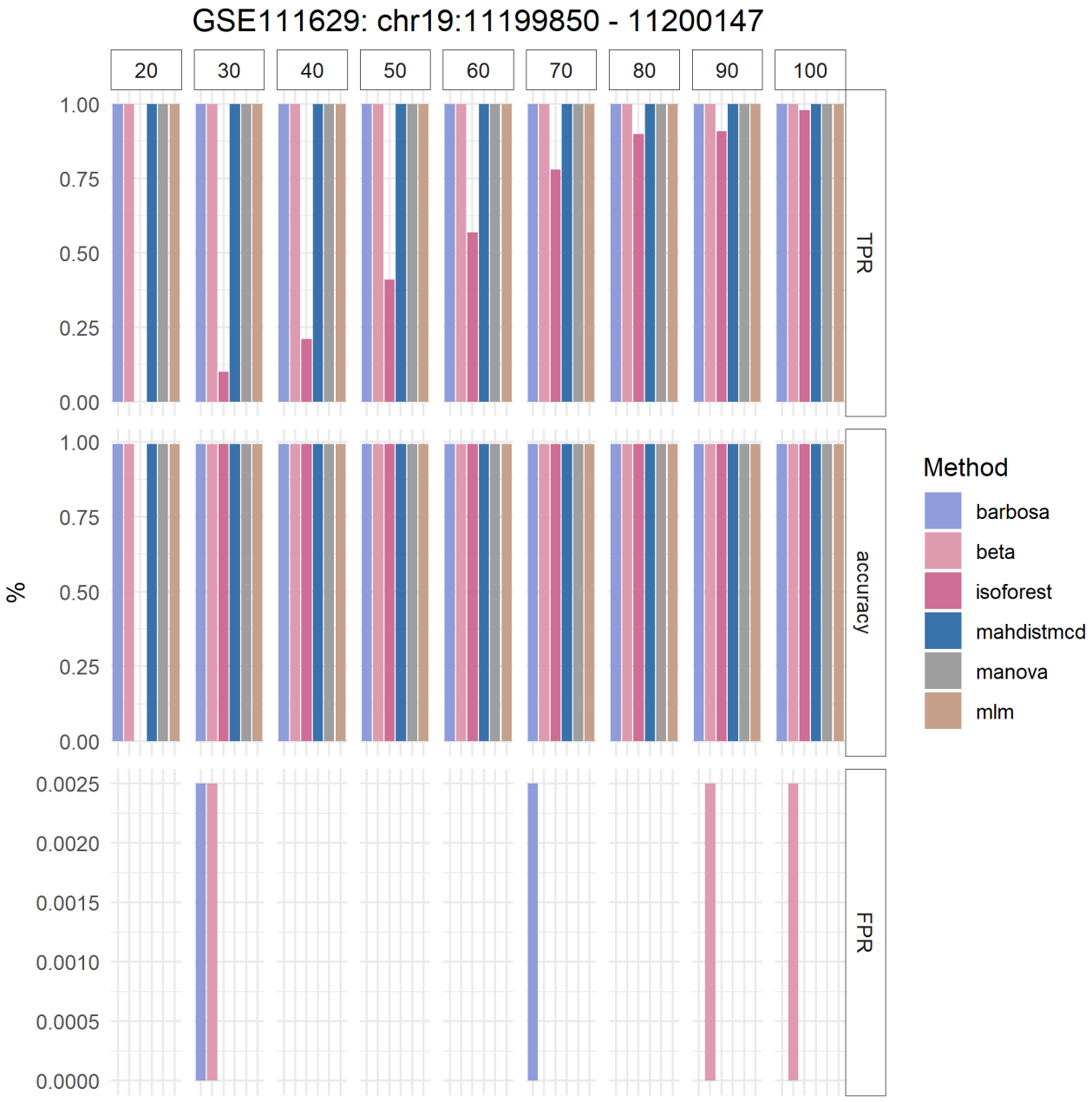

Figure 5: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:11199850-11200147

References

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Garg, Paras, Bharati Jadhav, Oscar L Rodriguez, Nihir Patel, Alejandro Martin-Trujillo, Miten Jain, Sofie Metsu, et al. 2020. "A Survey of Rare Epigenetic Variation in 23,116 Human Genomes Identifies Disease-Relevant Epivariations and CGG Expansions." *The American Journal of Human Genetics* 107 (4): 654–69.