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 onces (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 or excluding epivariations found (Garg et al. 2020).

The validated epimutations (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. Also, we define the regions selecting (1) entire chromosome and (2) 20 kb containing ≥ 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 ≥ 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 and accuracy $> 99\%$. Isolation forest detected the epivariations with similar accuracy but with a lower TPR (76%). These measures in barbosa method are 87% and 90% respectively. Nonetheless, qn approach identified the validated epimutations in 100% of the simulations but with very small accuracy (0.04%), which means a huge difference in the region width between the validated epimutation and the identified one by our method.

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 ≥ 3 GpGs. We compared each case sample individually against control samples. We observed that in both cohorts and for every approach the FPR was $< 0.01\%$.

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

method	TPR	accuracy	FPR
barbosa	0.8688889	0.8961111	0.0030556
isoforest	0.7622222	0.9960000	0.0213889
mahdistmcd	1.0000000	0.9960000	0.0202778
manova	0.9955556	0.9960000	0.0077778
mlm	1.0000000	0.9960000	0.0100000
qn	1.0000000	0.0380000	0.0163889

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

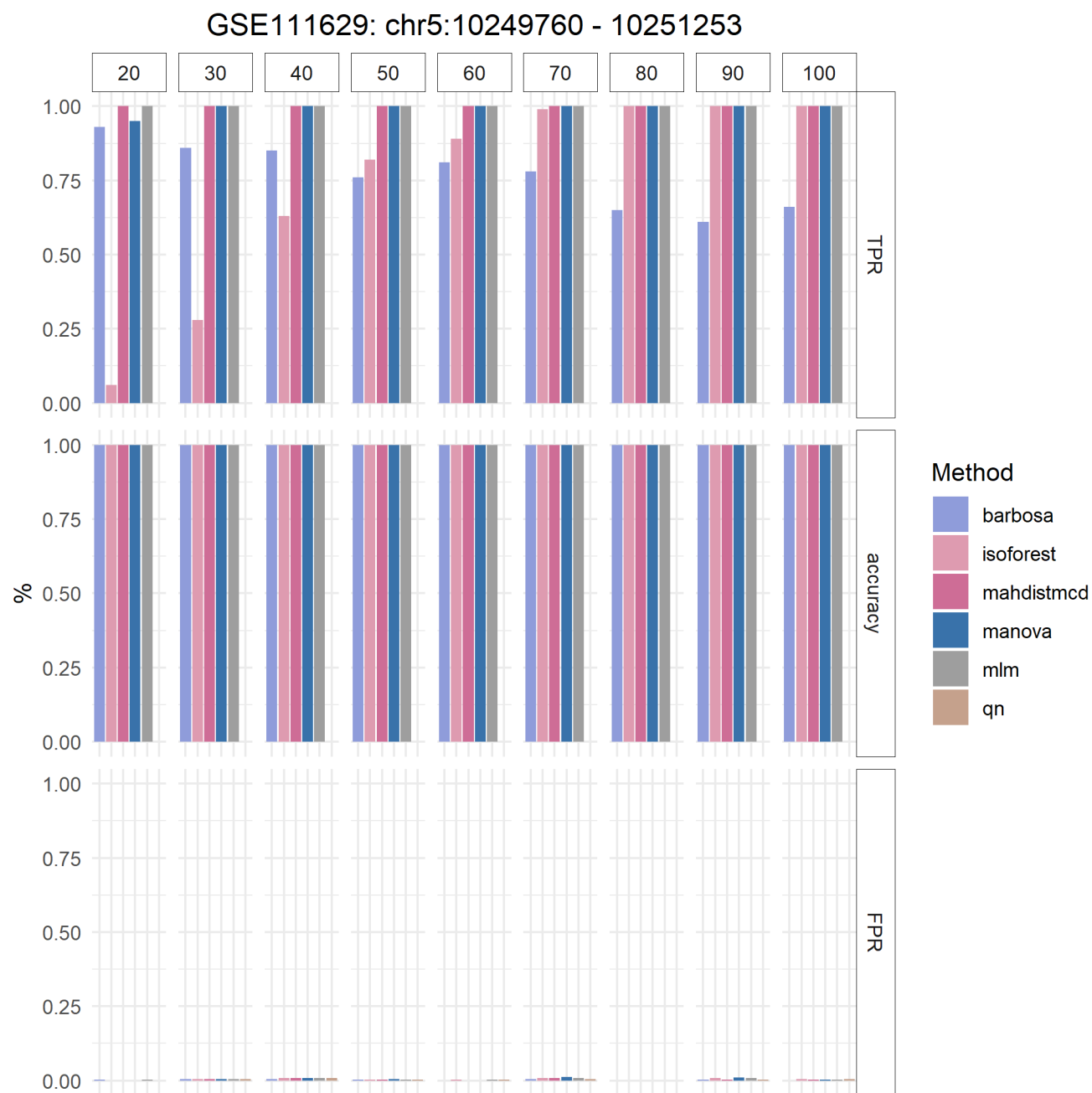| method	TPR	accuracy	FPR
barbosa	0.8369444	0.9395556	0.0025000
isoforest	0.6834722	0.9398889	0.0041667
mahdistmcd	1.0000000	0.9282500	0.0030556
manova	0.9986111	0.9282500	0.0047222
mlm	1.0000000	0.9282500	0.0041667
qn	1.0083333	0.0300000	0.0033333

Figure 1: epimutations performance for GSE51032 cohort detecting the epivariation located in chr5:10249760-10251253
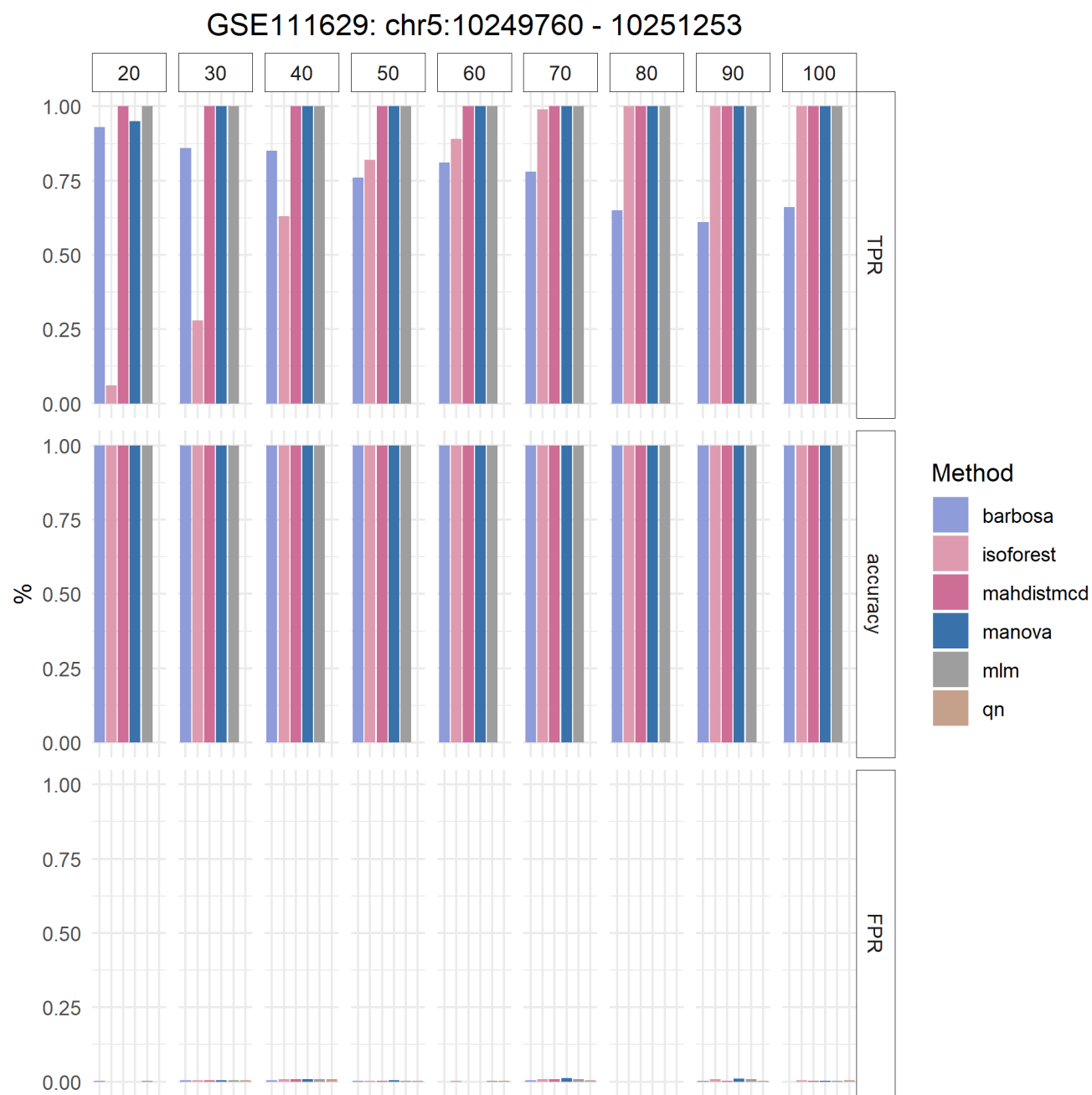
Figure 2: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:10249760-10251253
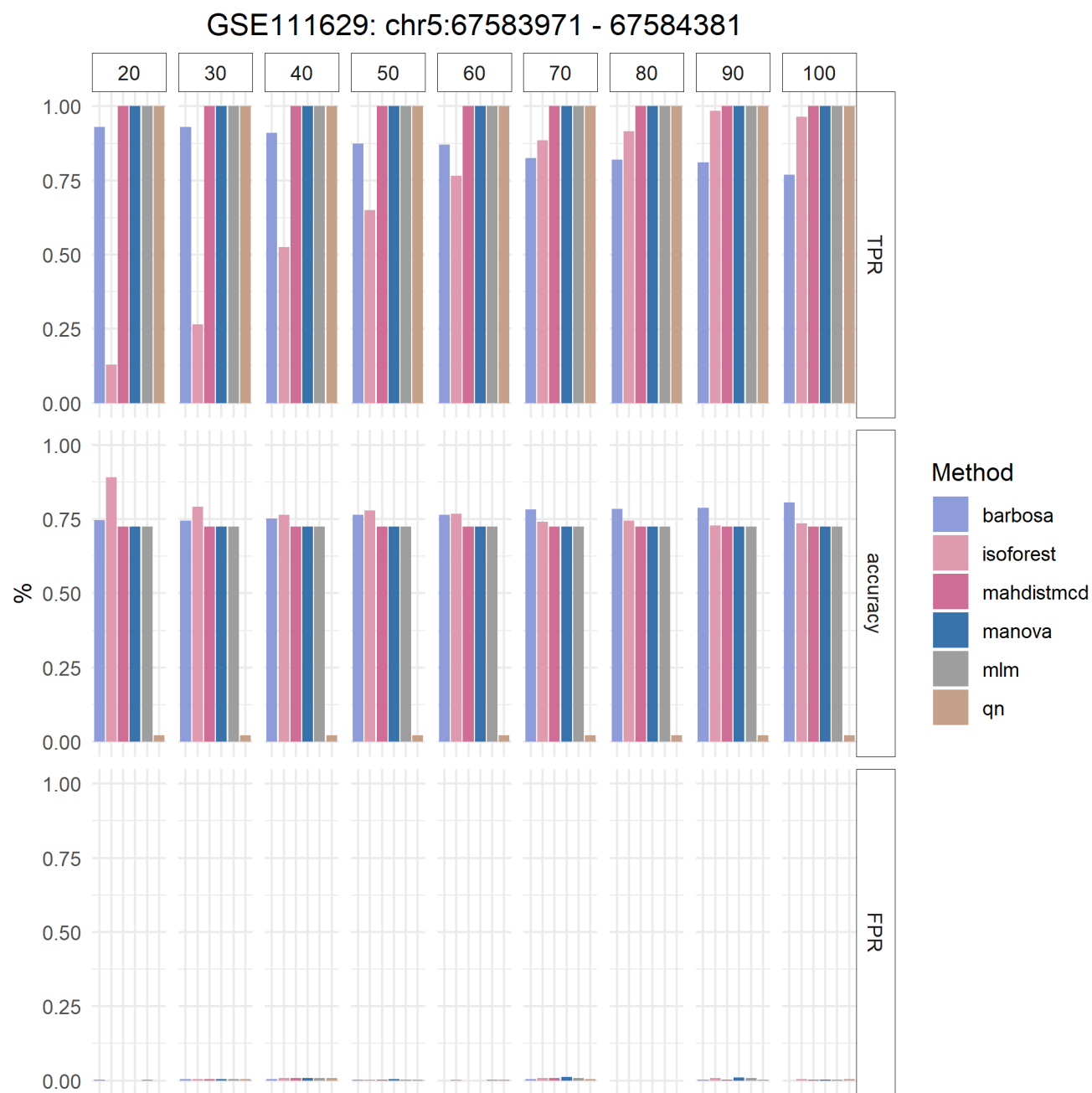
Figure 3: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:67583971-67584381

Figure 4: epimutations performance using GSE111629 cohort to detect the epivariation located in chr17:46018653-46019185
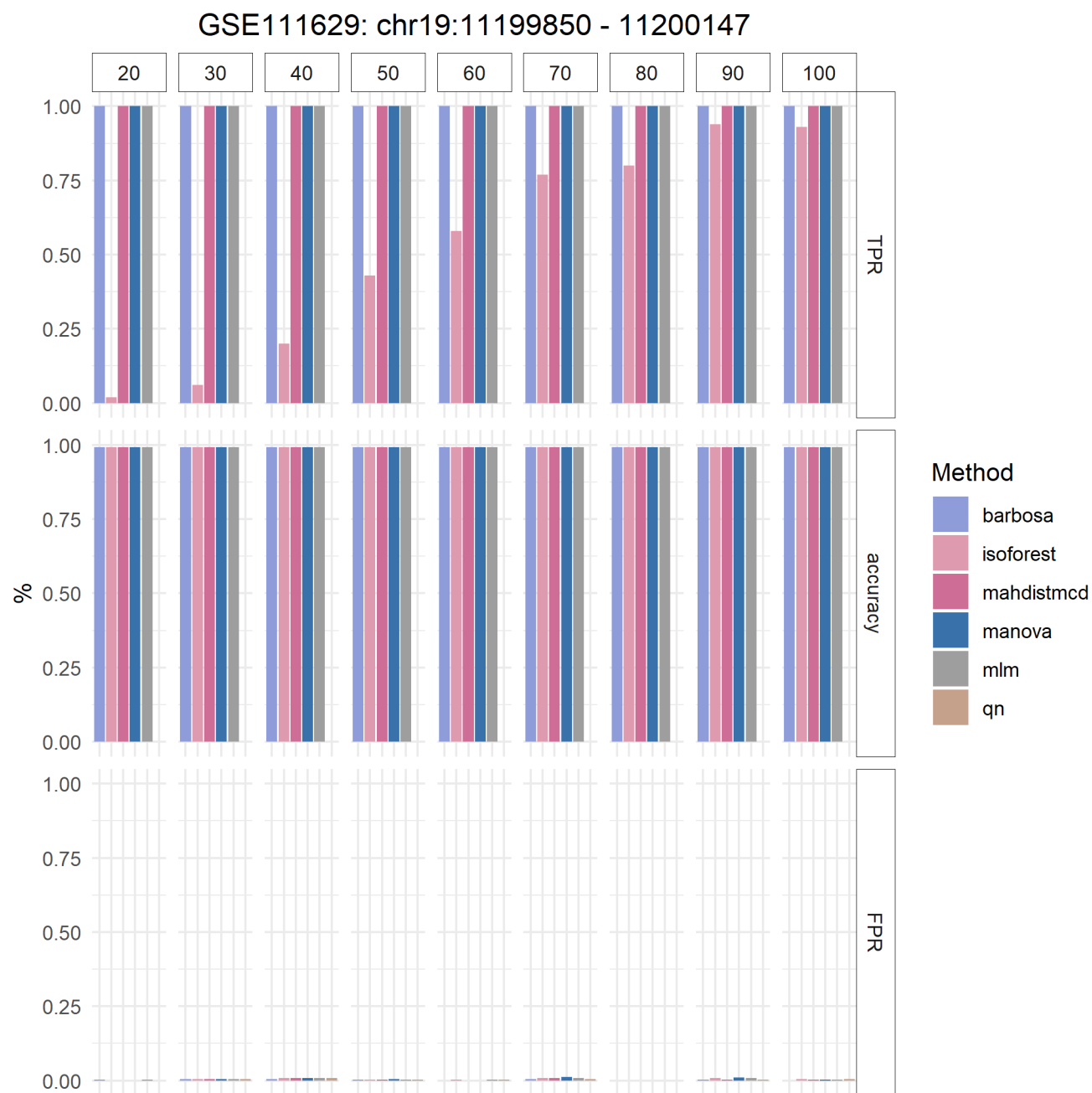
Figure 5: epimutations performance using GSE111629 cohort to detect the epivariation located in chr5:11199850-11200147

References

Aref-Eshghi, Erfan, Eric G. Bend, Samantha Colaiacovo, Michelle Caudle, Rana Chakrabarti, Melanie Napier, Lauren Brick, et al. 2019. "Diagnostic Utility of Genome-Wide DNA Methylation Testing in Genetically Unsolved Individuals with Suspected Hereditary Conditions." *The American Journal of Human Genetics.* https://doi.org/https://doi.org/10.1016/j.ajhg.2019.03.008.

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.