Methods for DMR analysis

- B. Data defined regions
  - *ProbeLasso* R package
    - https://bioconductor.org/packages/release/bioc/html/ChAMP.html
  - *DMRcate* R package
    - http://bioconductor.org/packages/release/bioc/html/DMRcate.html
  - bumphunting implemented in *minfi* R package
    - https://www.bioconductor.org/help/course-materials/2015/BioC2015/methylation450k.html
- Procedures
  - (1) computes p-values for each CpG
  - (2) identifies regions in the genome enriched with consecutive small p-values

Simulation study

1. 14 samples of normal samples with similar ages (GSE41169)
2. A-clustering software identified 3063 co-methylated clusters

- the clustering is performed by cycling through the sites, ordered by location, and merging together neighboring clusters (e.g. those within 200bp) if the distance measure (e.g. 1- spearman correlation) between them is smaller than a predefined threshold (e.g. 0.5)

A–clustering: a novel method for the detection of co–regulated methylation regions, and regions associated with exposure FREE

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Author Notes

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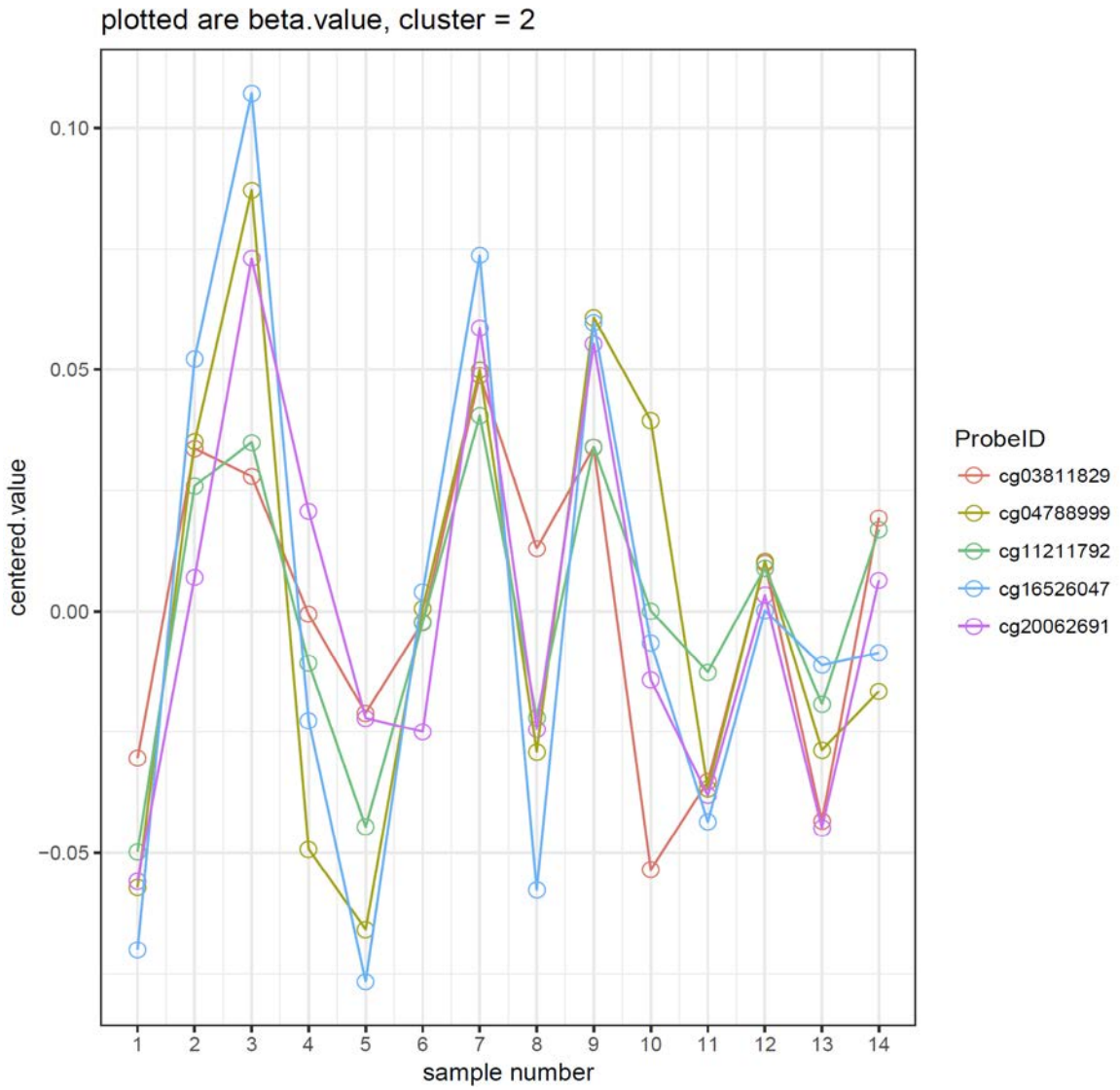

plotted are beta.value, cluster = 2
0.10
0.05
0.00
−0.05
centered.value
1
2
3
4
5
6
7
8
9
10
11
12
13
14
sample number
ProbeID
cg03811829
cg04788999
cg11211792
cg16526047
cg20062691

Simulation Study

3. Choose 500 random clusters
4. For each cluster, randomly divide samples into 2 groups
5. Compare group means, increase beta values in the group with higher mean by $\mu = \{0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4)$
6. Repeat 5 times

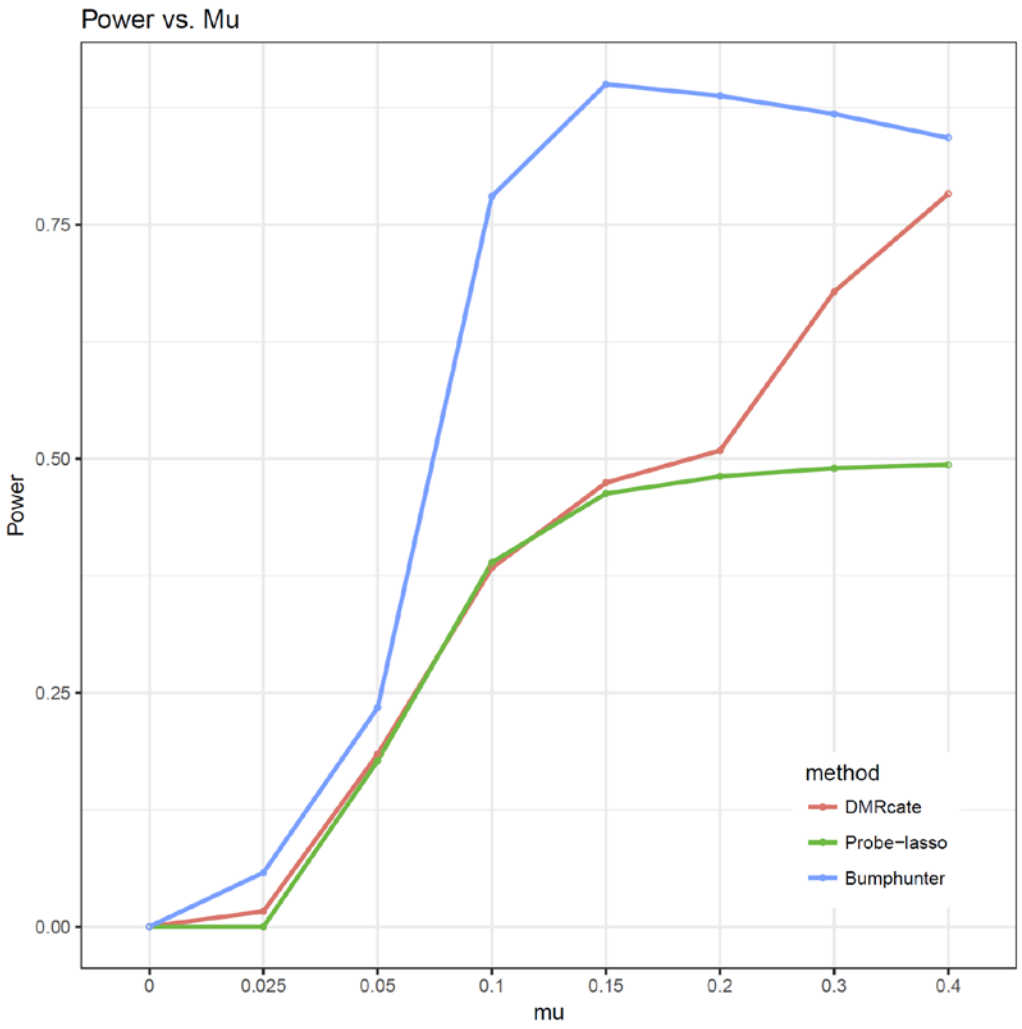
	actual positive	actual negative
predicted positive	TP	FP
predicted negative	FN	TN

(a) Confusion Matrix

A total of 40 simulation datasets

= 8 values for μ $\times$ 5 repetitions

Comparison of 3 type B methods - Power



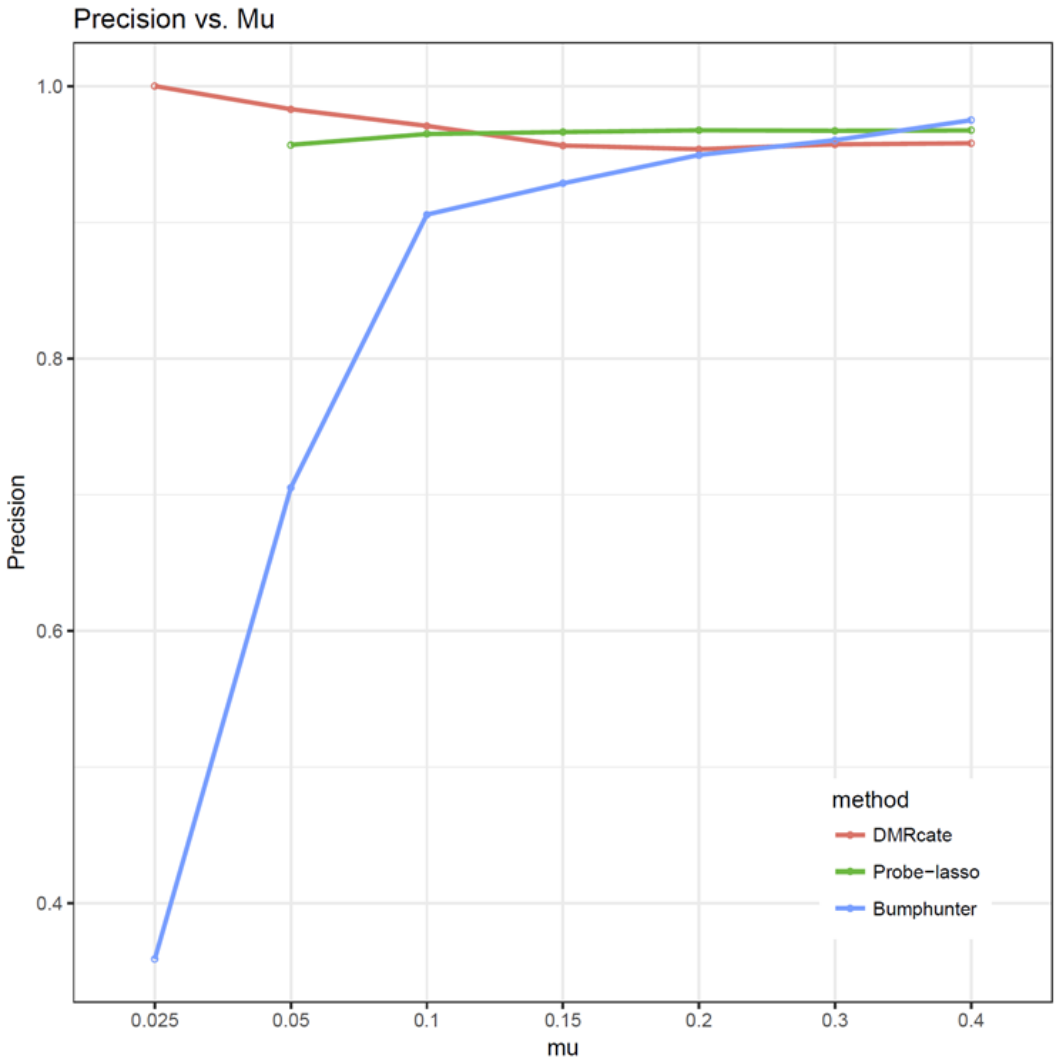
However, bumphunter is a permutation based method, very memory intensive.

Took about 8 min for 14 samples using parallel computing with 18 cores on a windows machine with 64G memory.

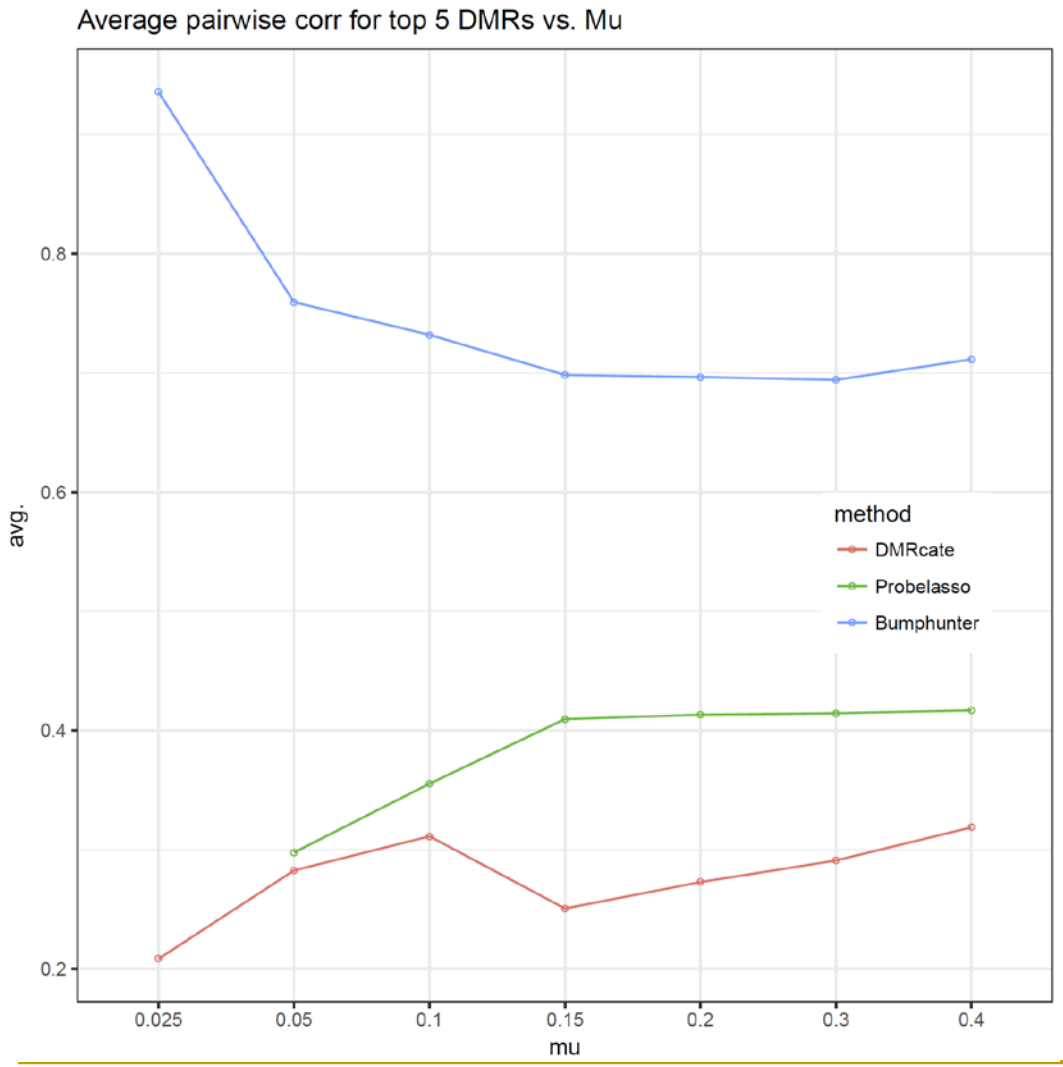
The other two methods took about 0.5 min without parallel computing.

Power = Probability (Predicted Positive | Actual Positive)

Comparison of 3 type B methods - Precision



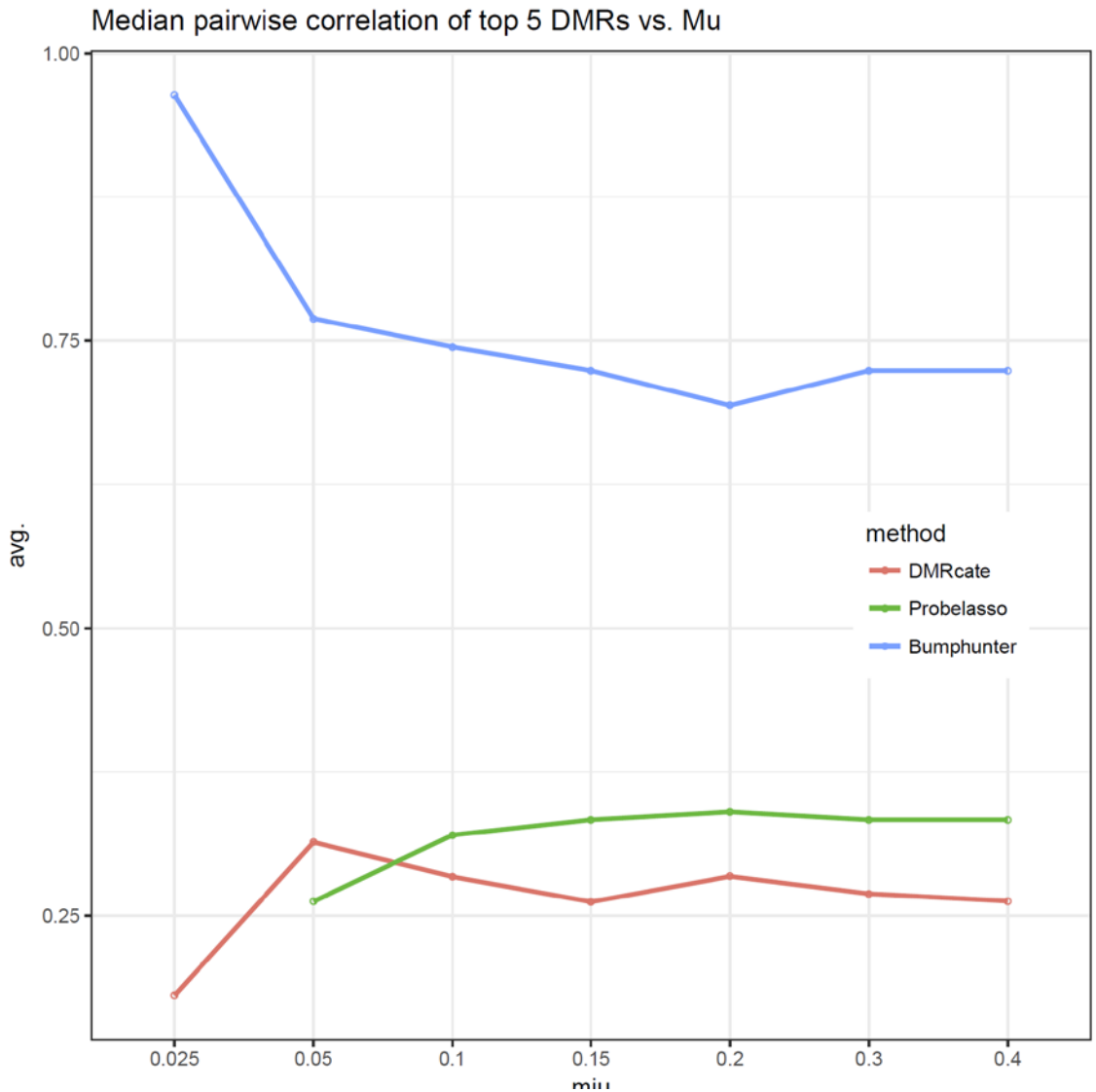
Precision = Probability (Actual Positive | Predicted Positive)

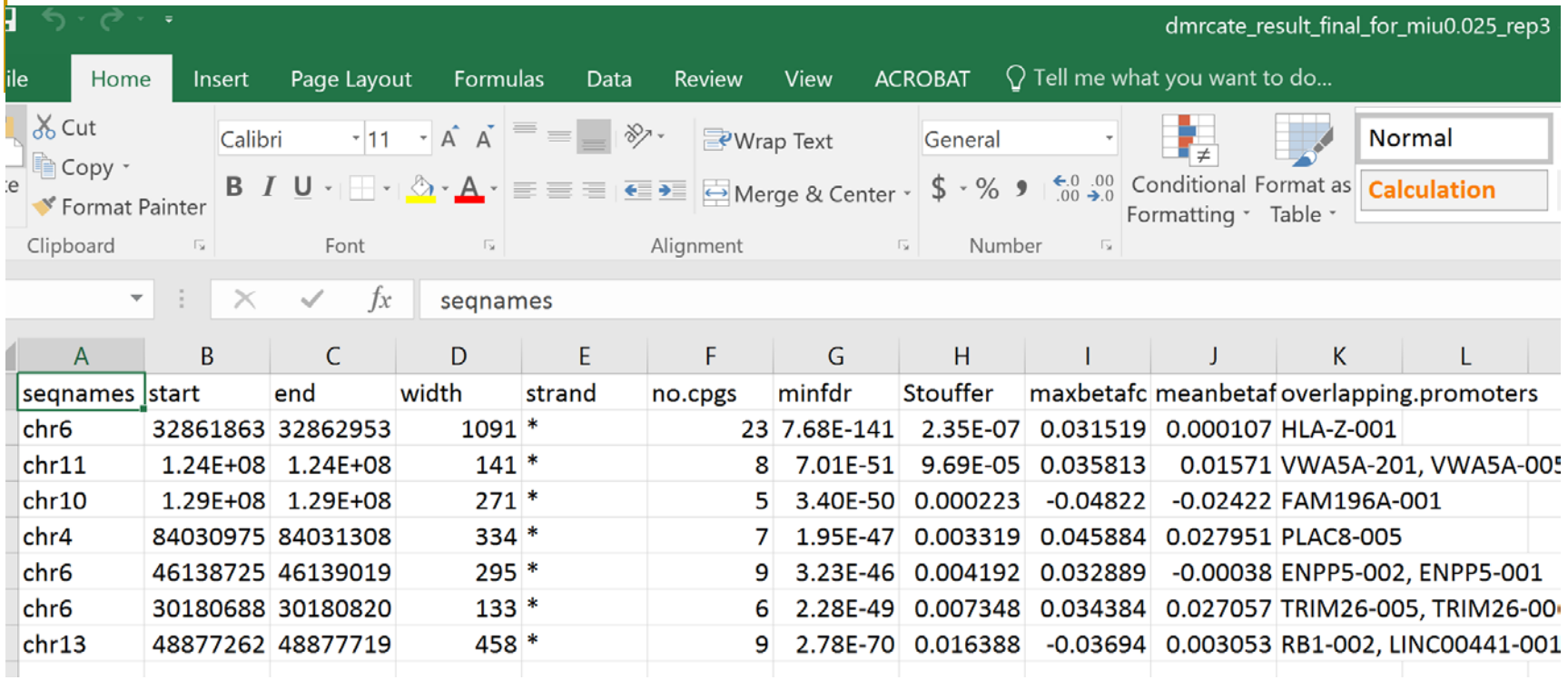


Take 5 most significant DMRs found by a method

extract pairwise correlations between cpgs within each DMR

Take average


Median pairwise correlation of top 5 DMRs vs. Mu
1.00
0.75
0.50
0.25
avg.
0.025
0.05
0.1
0.15
0.2
0.3
0.4
miu
method
DMRcate
Probelasso
Bumphunter



A	B	C	D	E	F	G	H	I	J	K	L
seqnames	start	end	width	strand	no.cpgs	minfdr	Stouffer	maxbetafc	meanbetaf	overlapping.promoters	
chr6	32861863	32862953	1091	*	23	7.68E-141	2.35E-07	0.031519	0.000107	HLA-Z-001	
chr11	1.24E+08	1.24E+08	141	*	8	7.01E-51	9.69E-05	0.035813	0.01571	VWA5A-201, VWA5A-005	
chr10	1.29E+08	1.29E+08	271	*	5	3.40E-50	0.000223	-0.04822	-0.02422	FAM196A-001	
chr4	84030975	84031308	334	*	7	1.95E-47	0.003319	0.045884	0.027951	PLAC8-005	
chr6	46138725	46139019	295	*	9	3.23E-46	0.004192	0.032889	-0.00038	ENPP5-002, ENPP5-001	
chr6	30180688	30180820	133	*	6	2.28E-49	0.007348	0.034384	0.027057	TRIM26-005, TRIM26-00	
chr13	48877262	48877719	458	*	9	2.78E-70	0.016388	-0.03694	0.003053	RB1-002, LINC00441-001	

DMRcate result – note that this only includes information on a subset of genes in the genome

Comments on type B methods

- All methods did well in terms of precision when effect size (μ) is moderate (i.e. > 0.1)
- Bumphunter had highest power, but was also most memory intensive
- DMRs detected by bumphunter also had the highest level of co-methylation
- Recommend bumphunter for datasets with moderate to large effect sizes (μ > 0.1)

Comments on type A & B approaches

- In contrast to gene expression, methylation regions are often poorly defined, so approaches in *B* (data defined regions) might have more power

- On the other hand, approaches in *A* (user defined regions) might be better suited for mega or integrative analysis