Accurate inference of DNA methylation data:
Statistical challenges lead to biological insights

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CFCE Seminar
Dana-Farber Cancer Institute
15 February 2019

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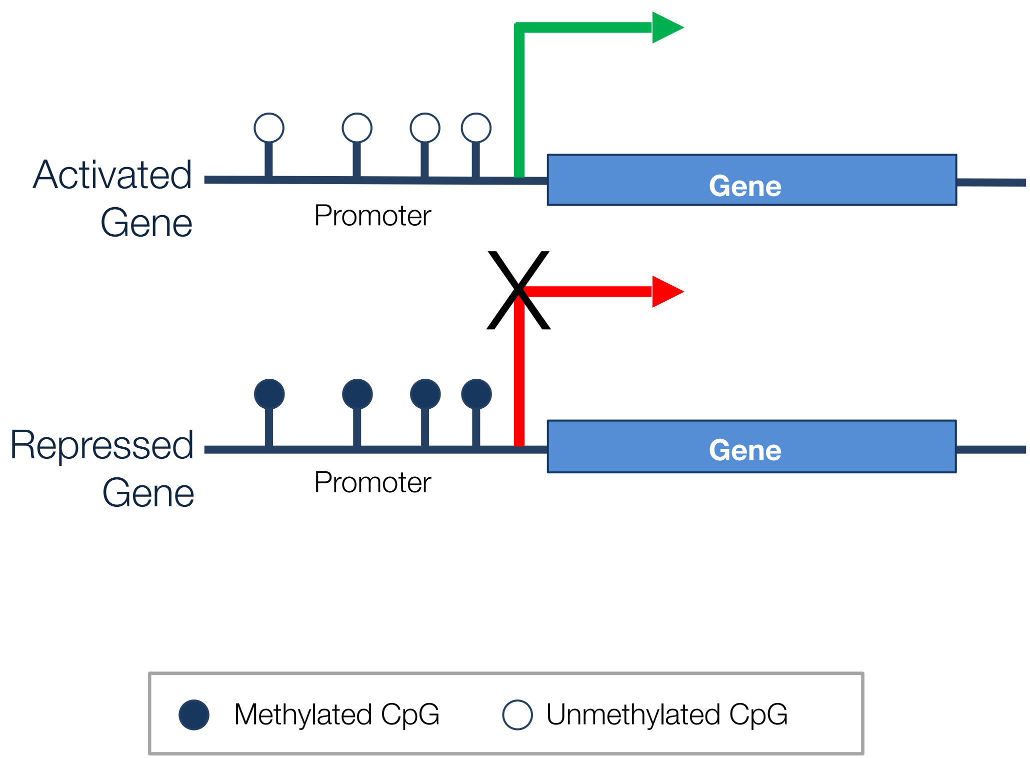
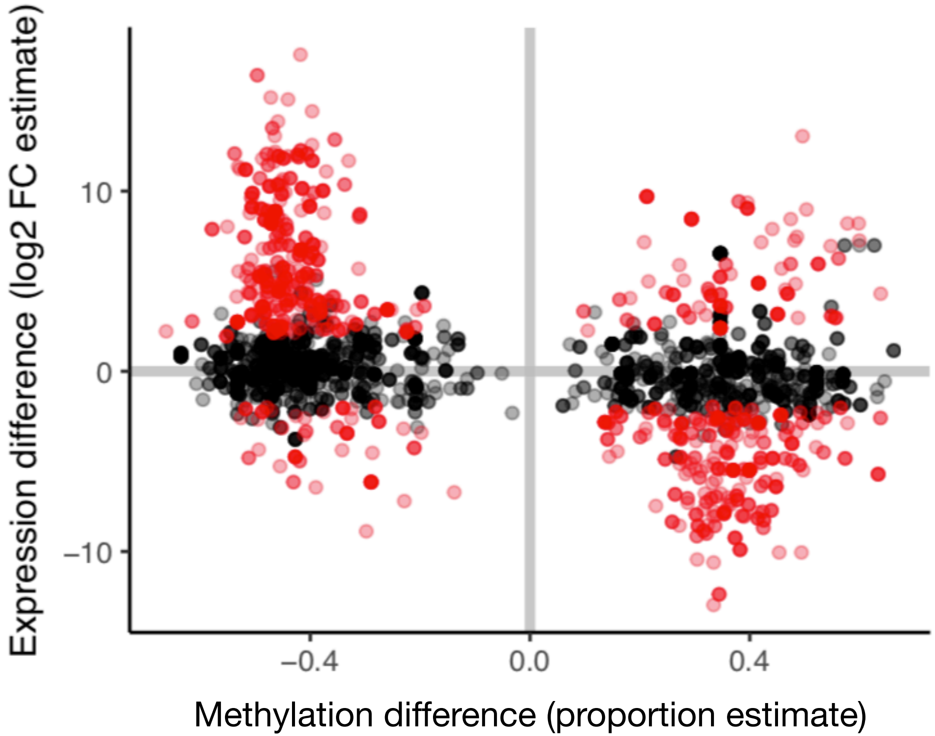
Role of DNA methylation in transcriptional regulation



Correlation or causation?



Korthauer et al., 2018 (*Biostatistics*)

First genome-wide study of causality

New Results – September 2017

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Frequent lack of repressive capacity of promoter DNA methylation identified through genome-wide epigenomic manipulation

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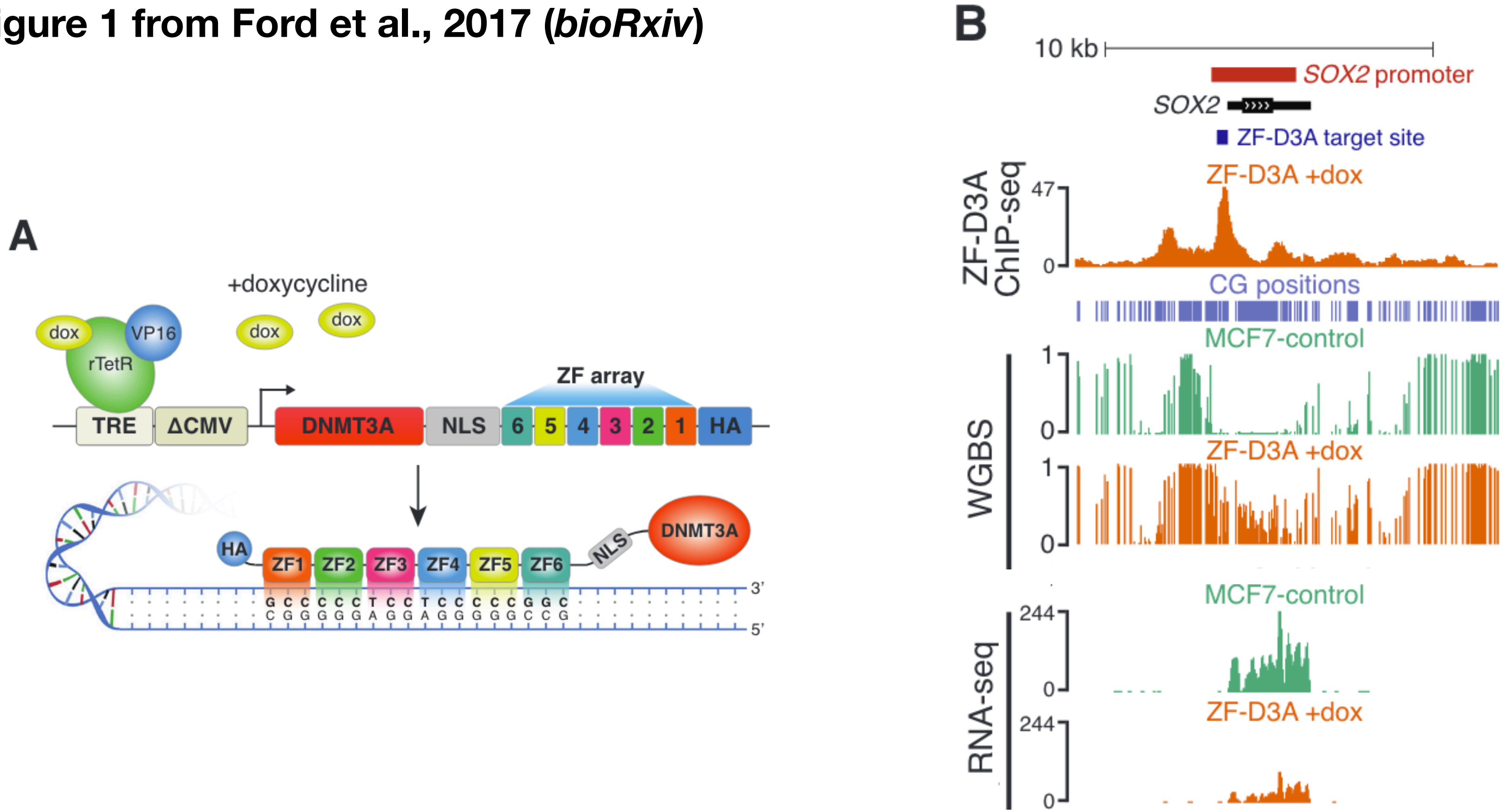
“promoter DNA methylation is **not generally sufficient** for transcriptional inactivation”

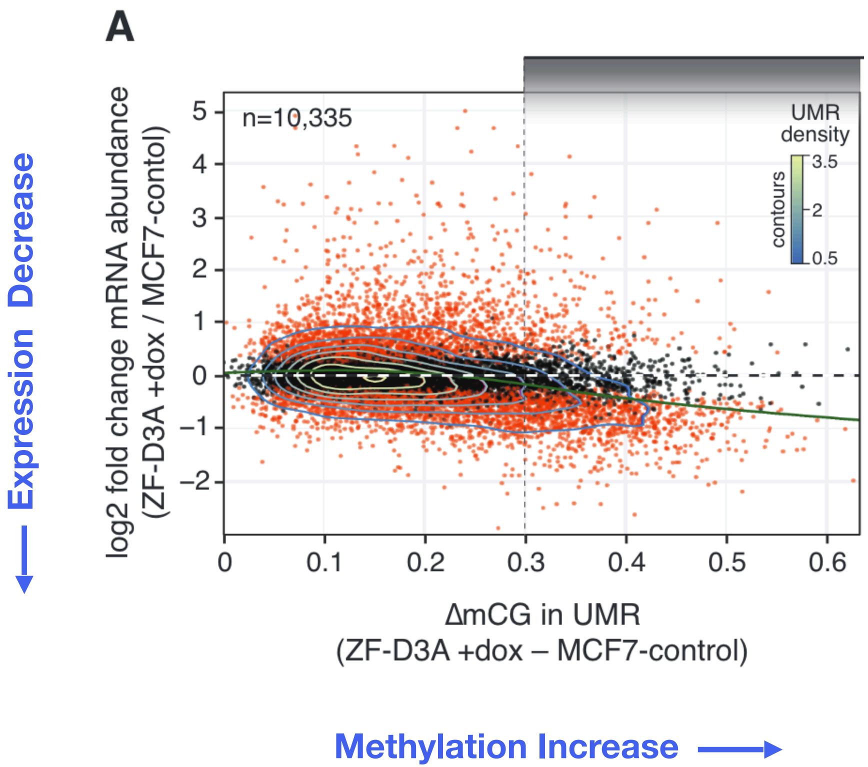
Forcible methylation of promoters

Figure 1 from Ford et al., 2017 (*bioRxiv*)



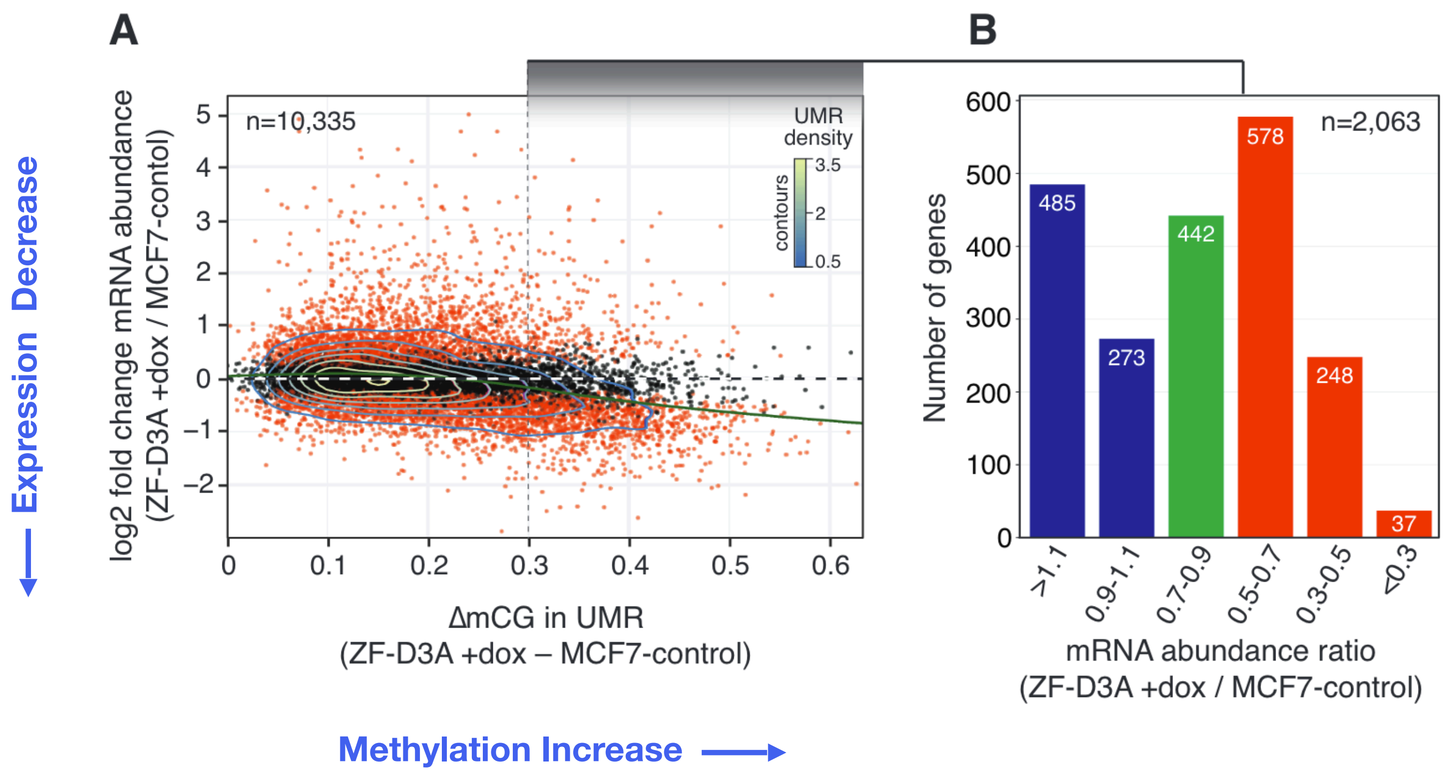
Conclusion: methylation not generally sufficient for gene repression

Figure 5 from Ford et al., 2017 (*bioRxiv*)



Conclusion: methylation not generally sufficient for gene repression

Figure 5 from Ford et al., 2017 (*bioRxiv*)



Statistical challenges

Challenges of methylation sequencing analysis

1. Small sample sizes

2. Region-level inference

3. Biological and spatial variability

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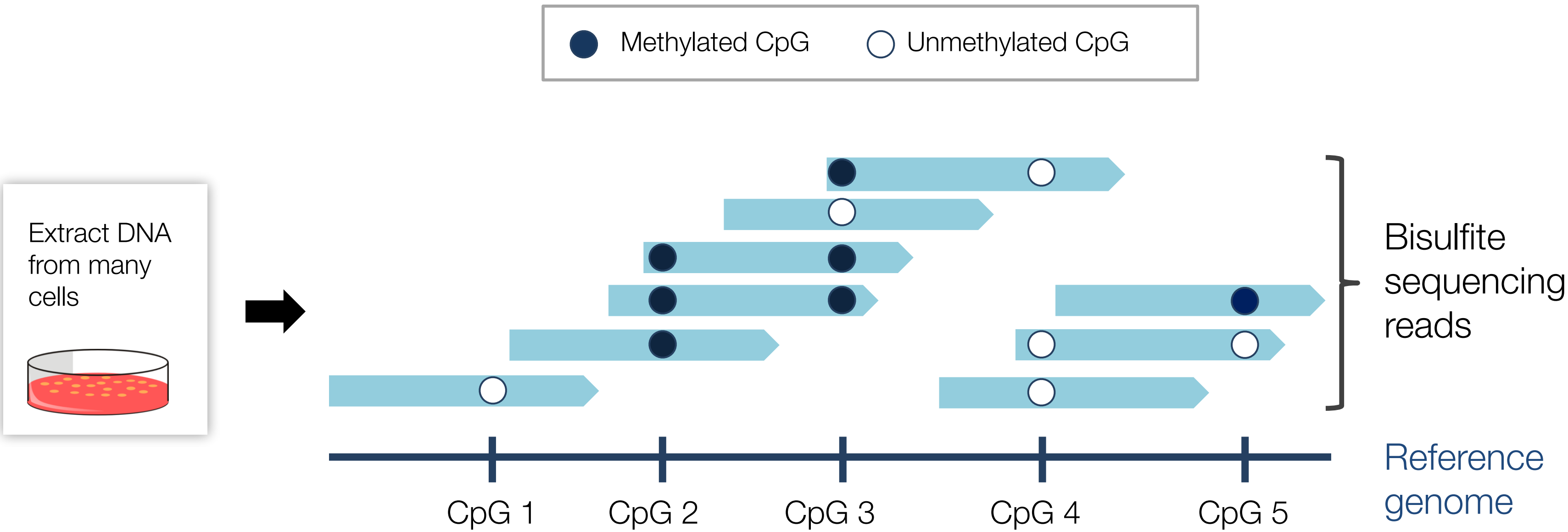
ENCODE

Whole genome bisulfite sequencing (WGBS)



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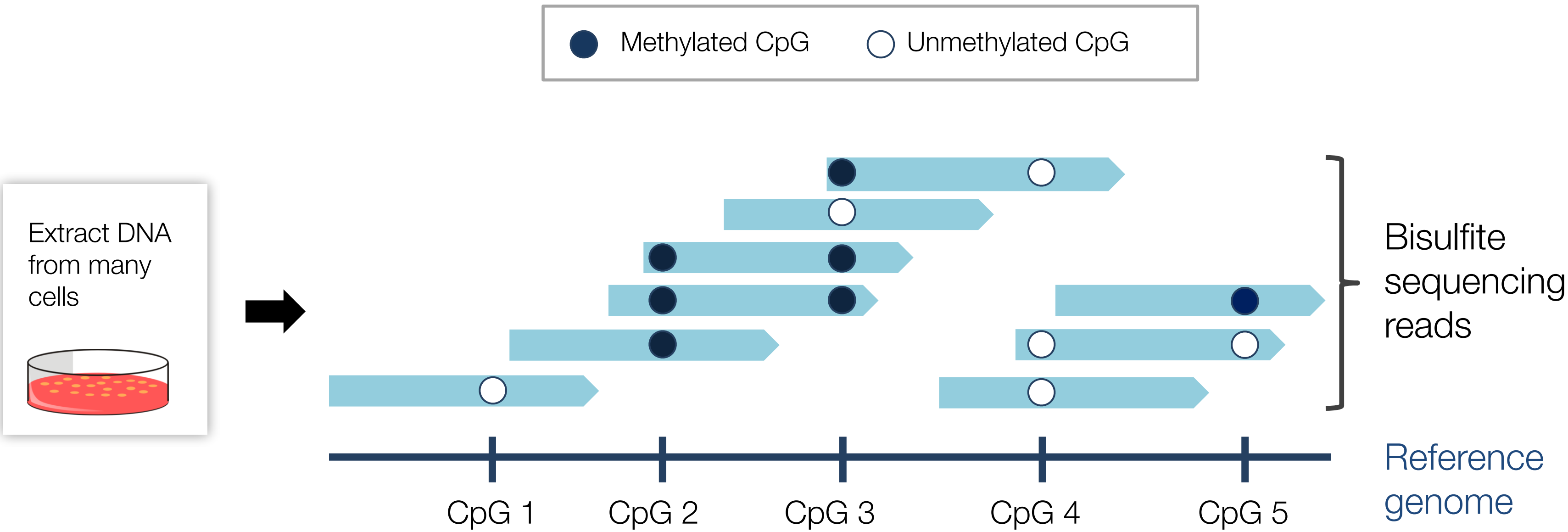
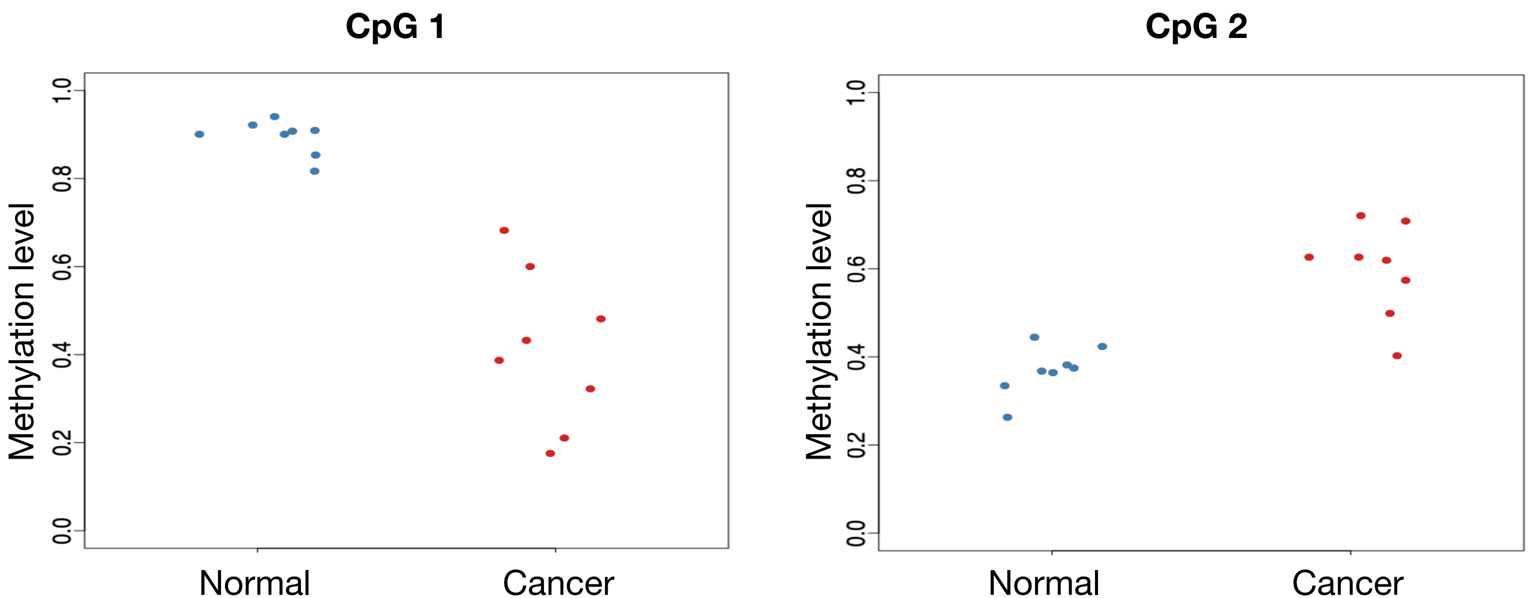


Methylated Count (M)	0	3	3	0	1
Coverage (N)	1	3	4	3	2
Proportion (M/N)	0	1	0.75	0	0.50

Methylation sequencing data

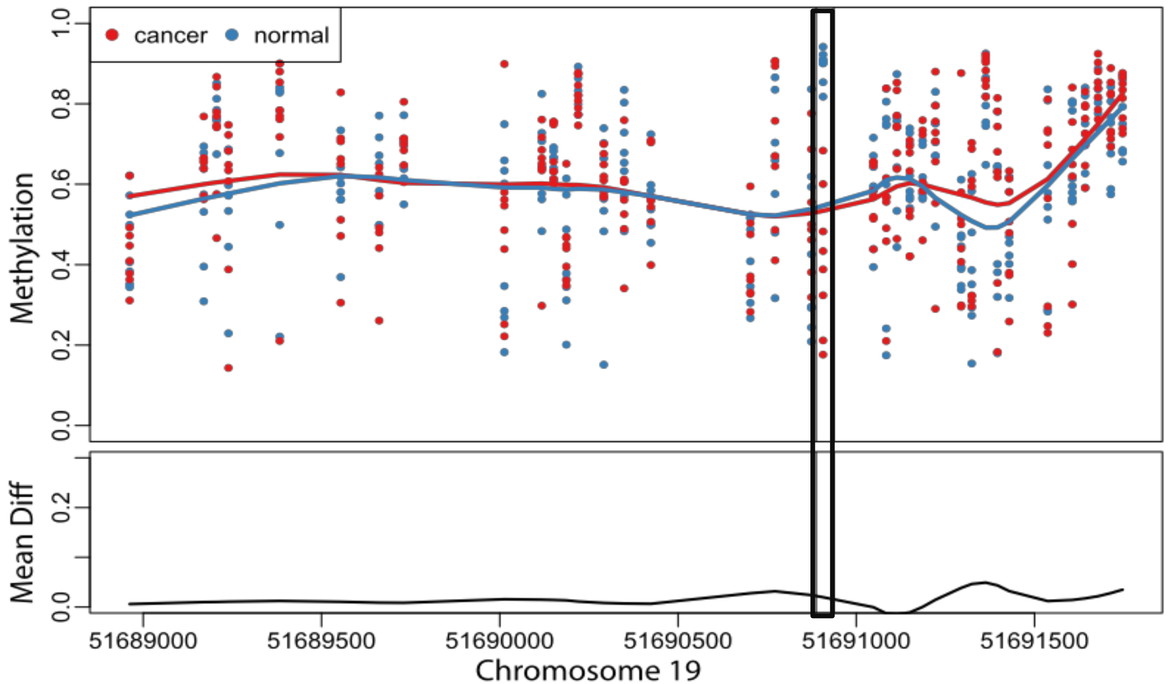
⚠️ WGBS cost ≈ WGS cost

Differential methylation of individual CpGs



Adapted from Jaffe et al., 2012 (*Int J Epidemiol*)

CpG 1



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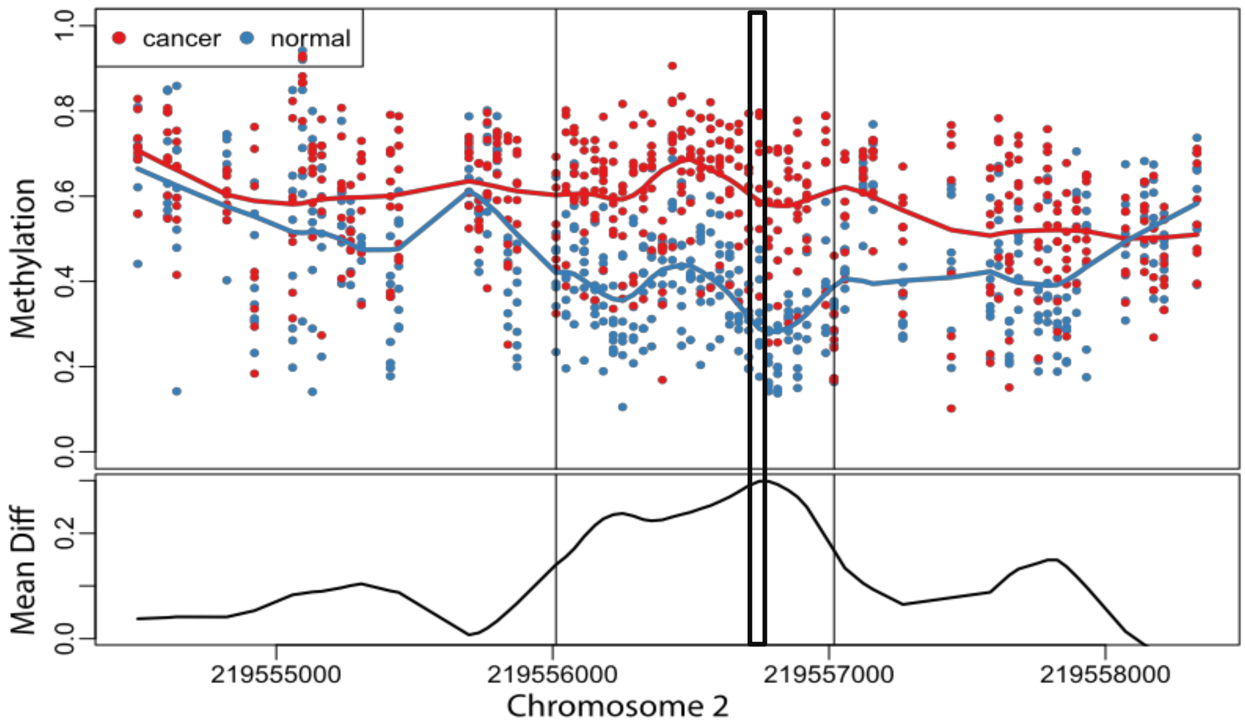
CpG 2



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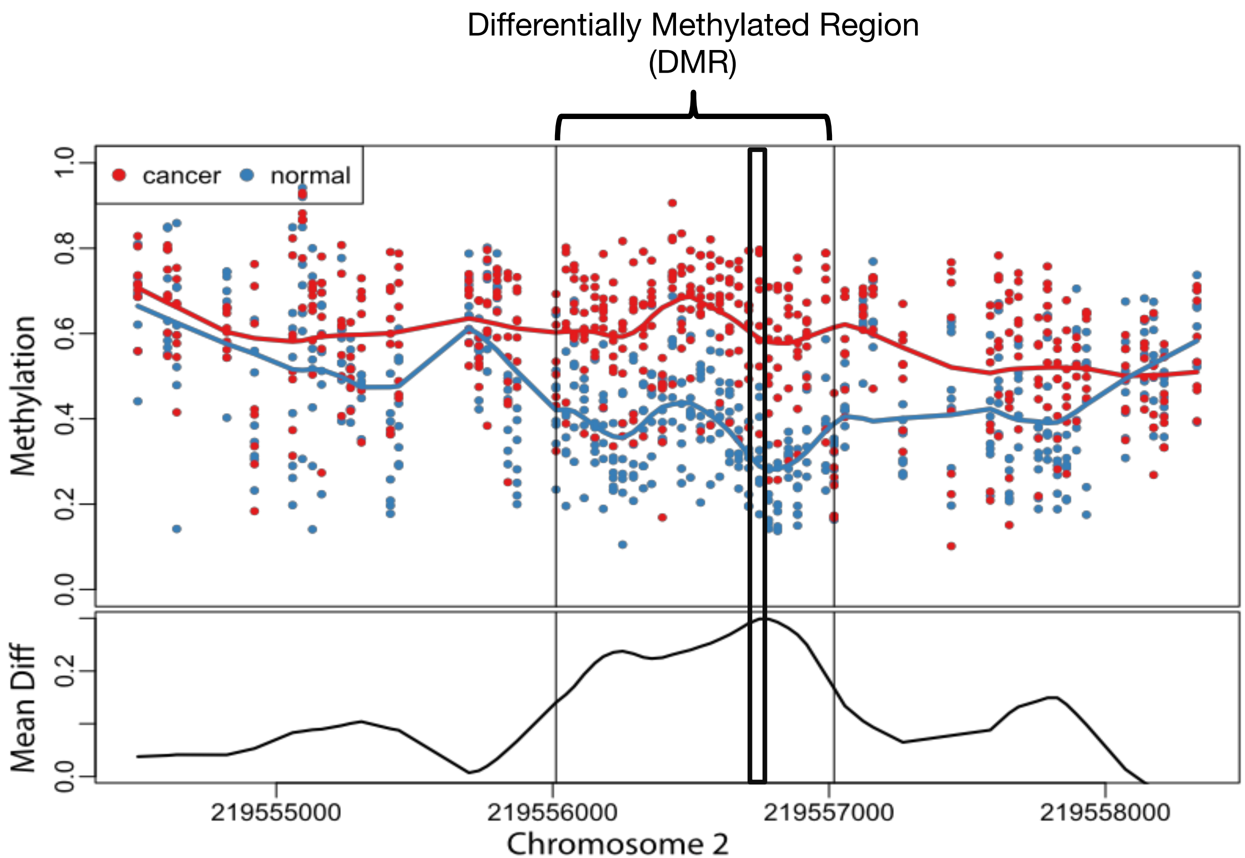

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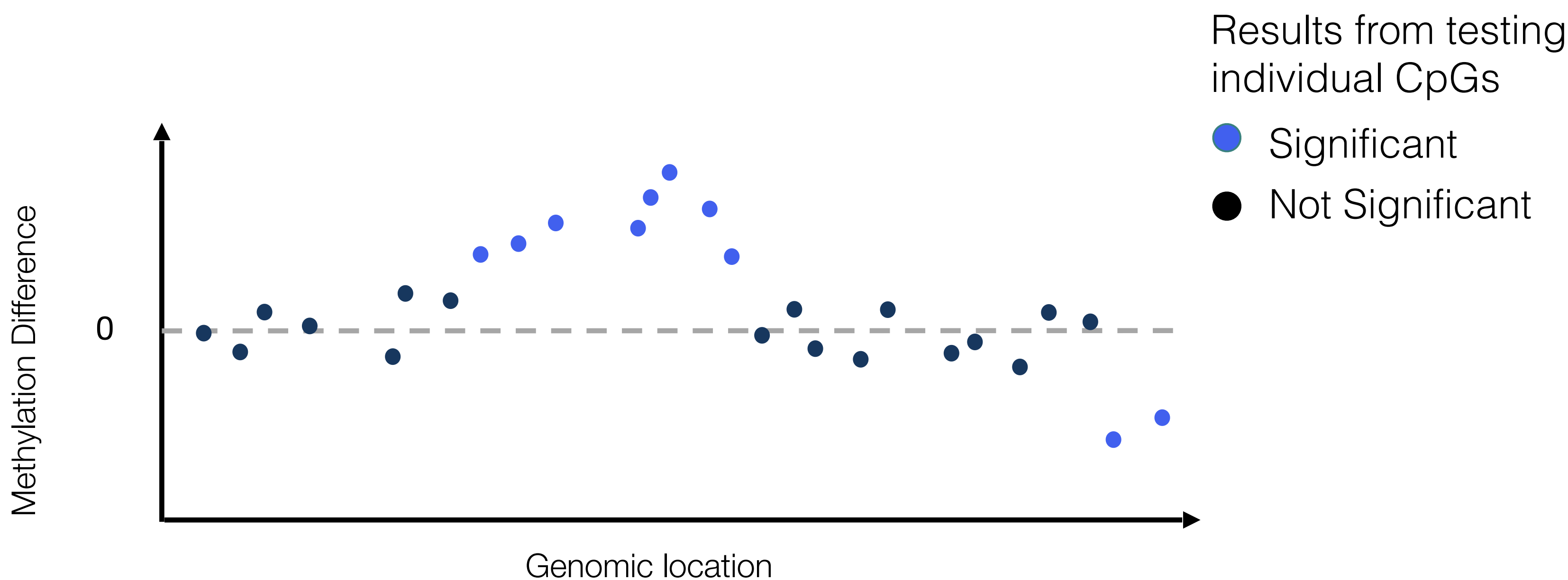
Previous methods: Grouping significant CpGs



Examples:

- Bsmooth (Hansen et al., 2012)
- DSS (Feng et al., 2014; Wu et al., 2015) – used by Ford et al.

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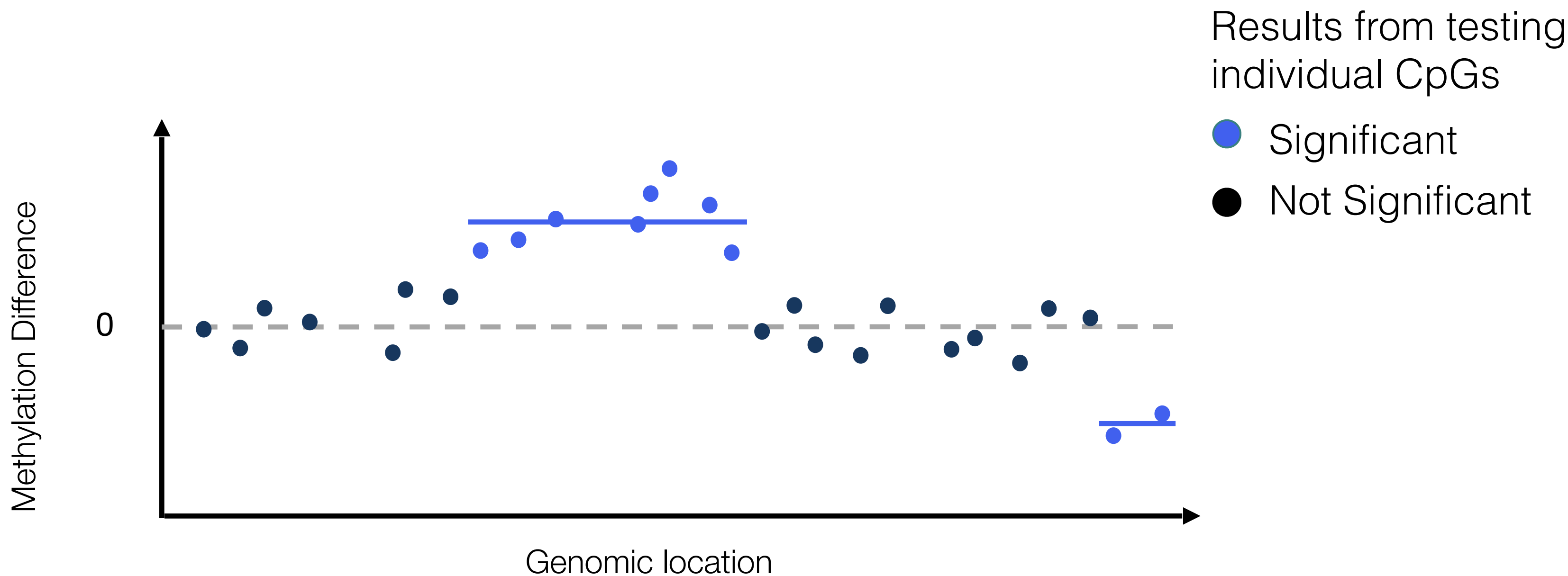

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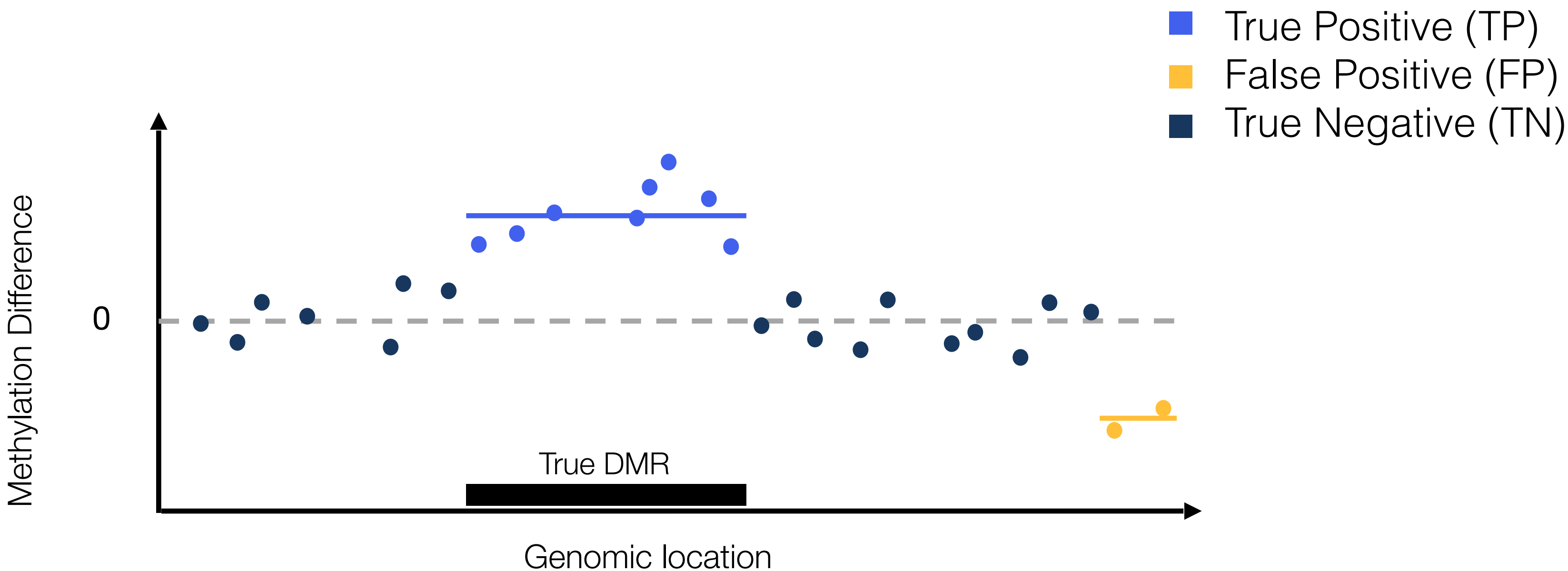
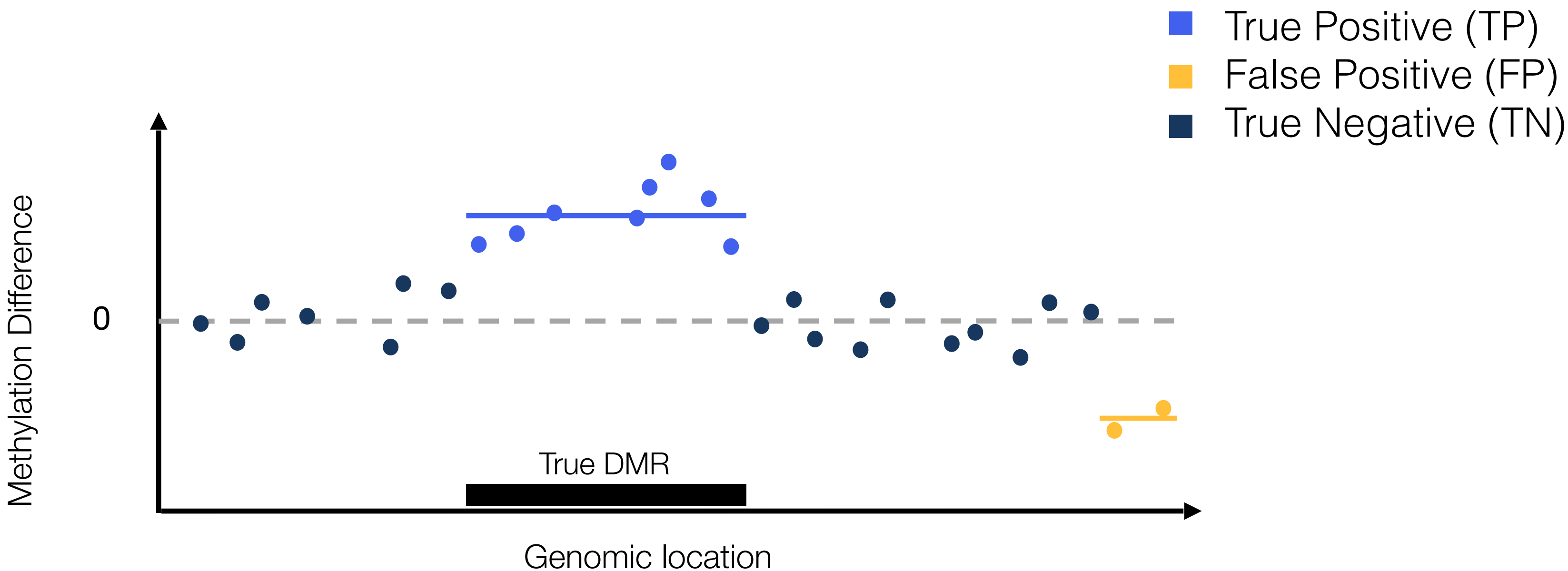
Error rate not controlled at the region level



False Discovery Rate (FDR) $= \mathrm{E}\left[\frac{\mathrm{FP}}{\mathrm{TP}+\mathrm{FP}}\right]$

Error rate not controlled at the region level



$$\text{False Discovery Rate (FDR)} = \mathrm{E}\left[\frac{\mathrm{FP}}{\mathrm{TP}+\mathrm{FP}}\right]$$

$$F\hat{D}R_{CpG} = \frac{2}{10} = 0.2$$

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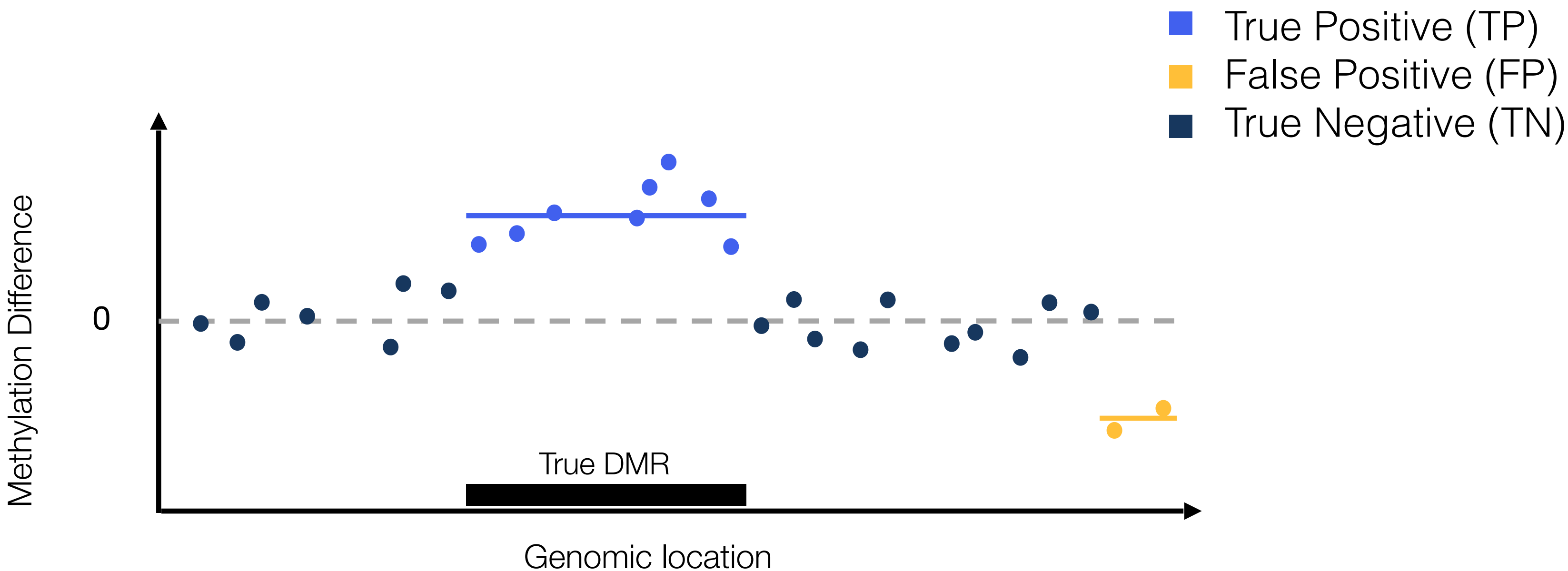
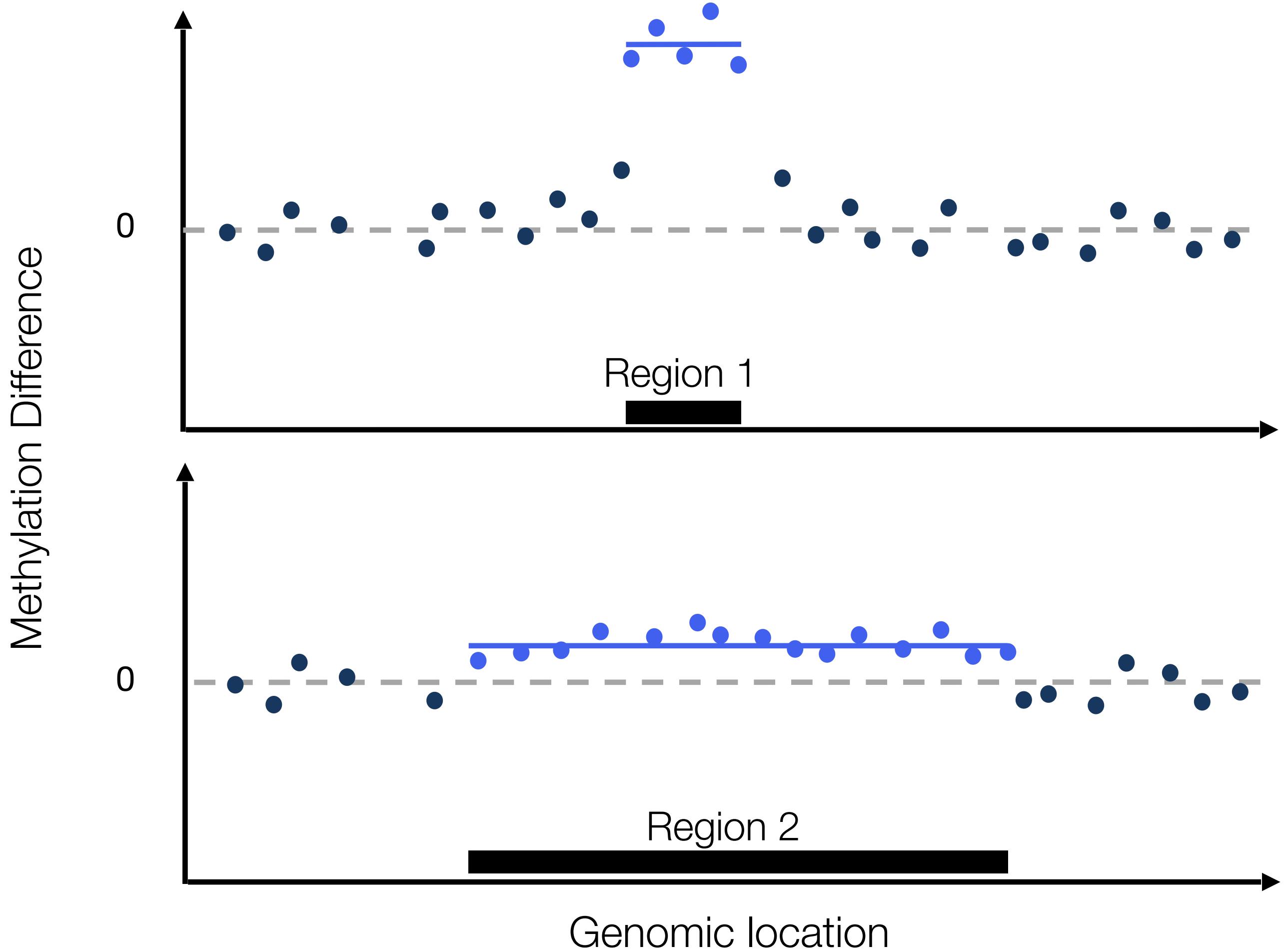


$$\text{False Discovery Rate (FDR)} = \mathrm{E}\left[\frac{\mathrm{FP}}{\mathrm{TP} + \mathrm{FP}}\right]$$

$$F\hat{D}R_{CpG} = \frac{2}{10} = 0.2 \quad vs \quad F\hat{D}R_{DMR} = \frac{1}{2} = 0.5$$

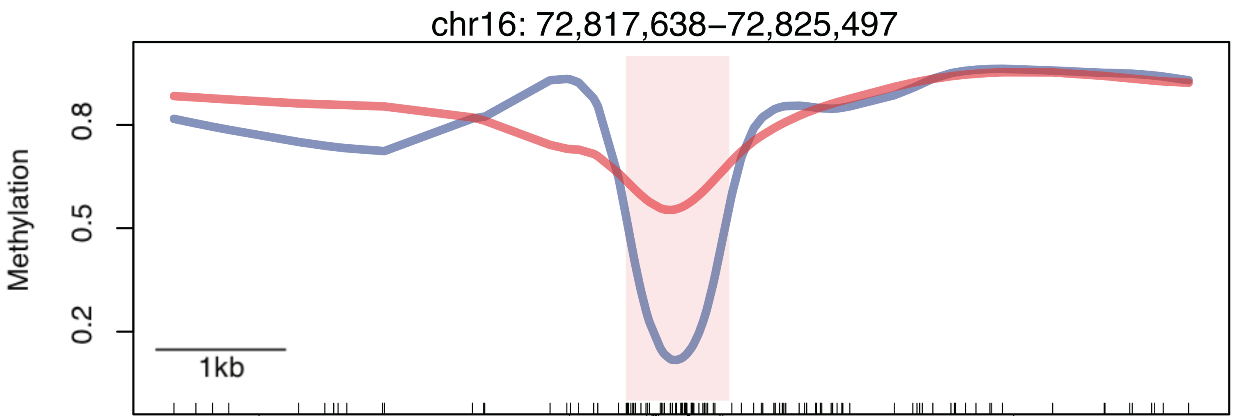
Spatial Variability



Prioritized by mean difference statistics

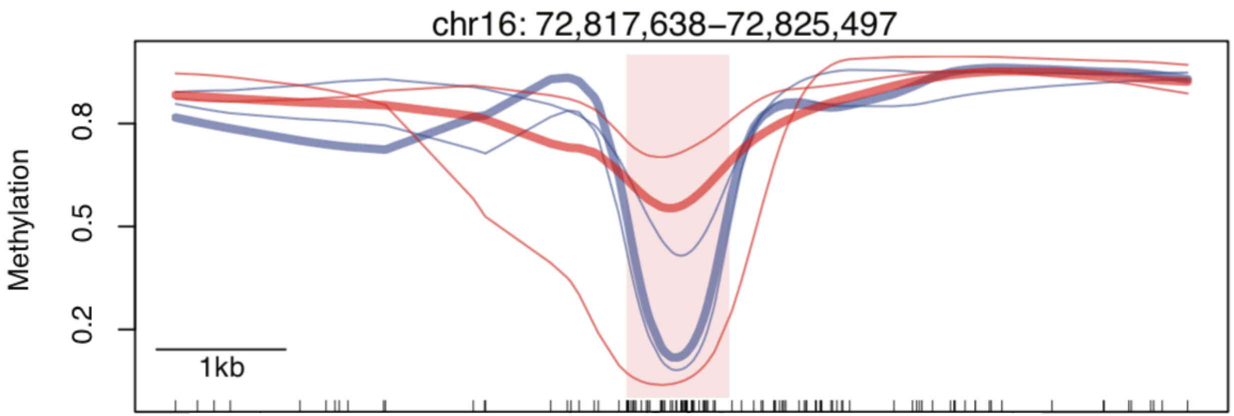
Prioritized by area (sum) statistics

Biological variability



Adapted from Hansen et al., 2011 (*Nature Genetics*)

Biological variability



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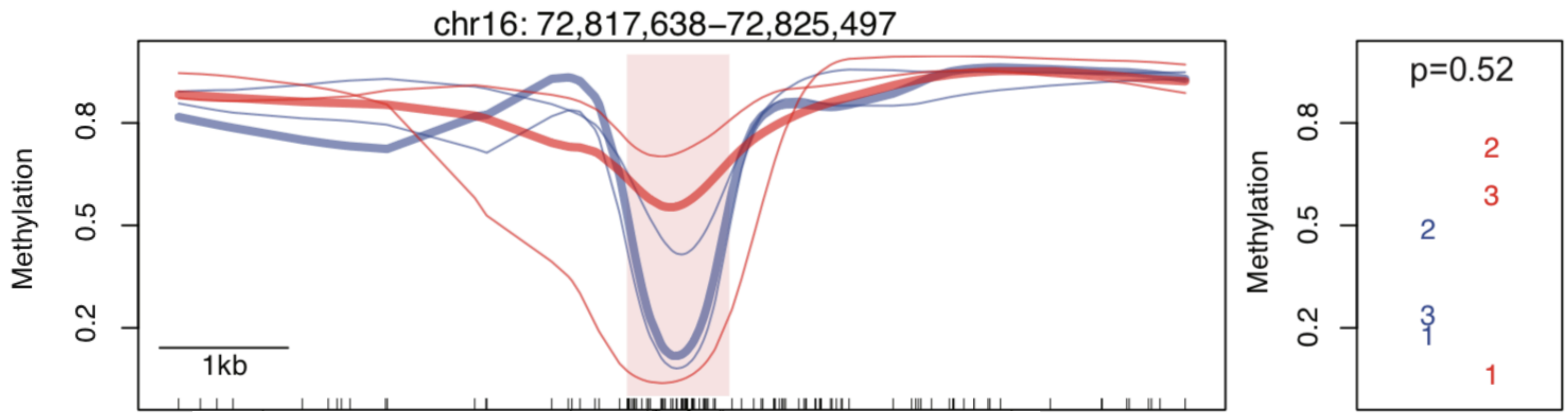

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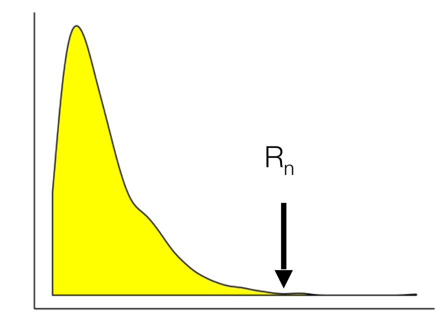
Methodology

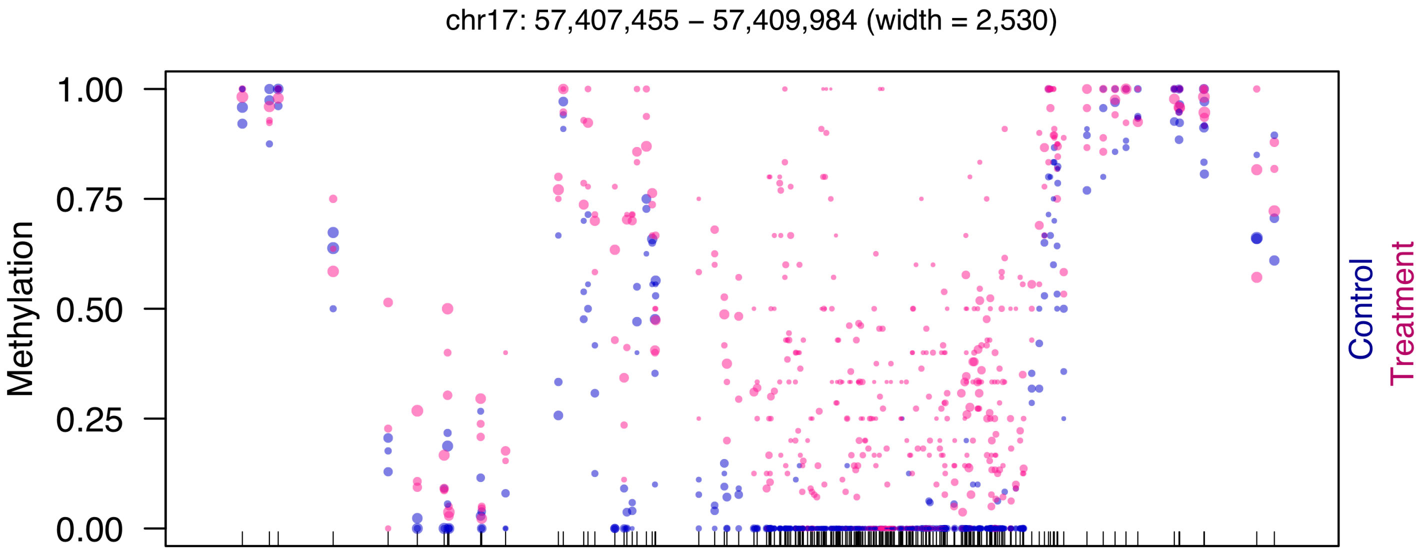
dmrseq: two-stage approach

1. Detect *de novo* candidate regions

2. Evaluate statistical significance



dmrseq: (1) Detect *de novo* candidate regions



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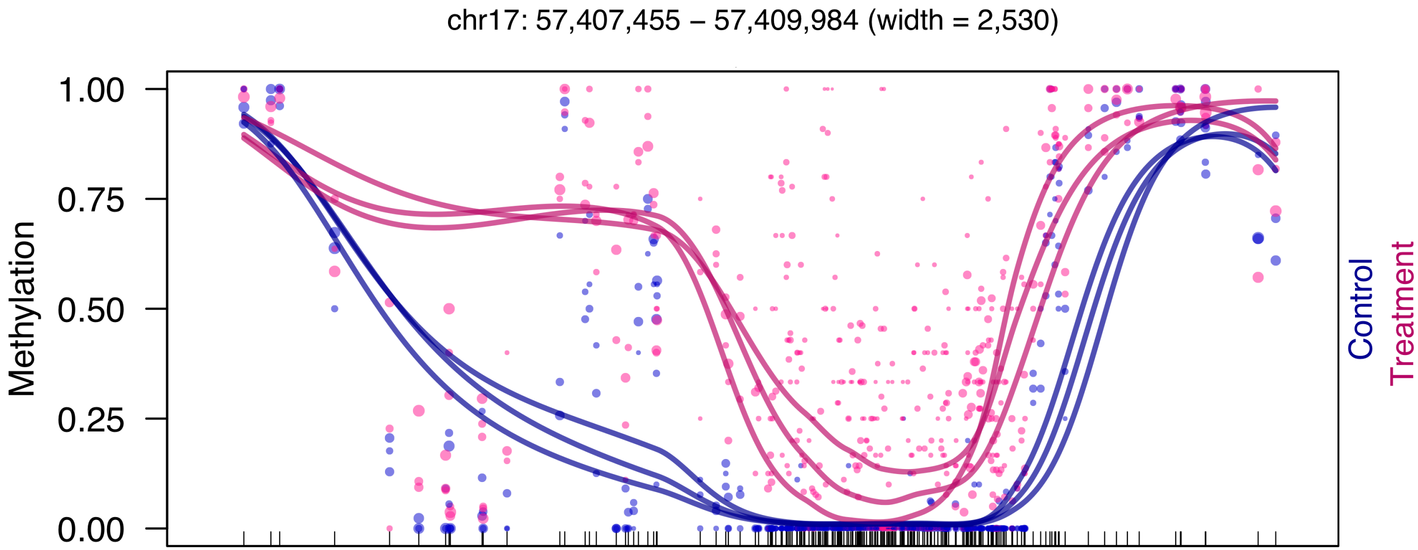

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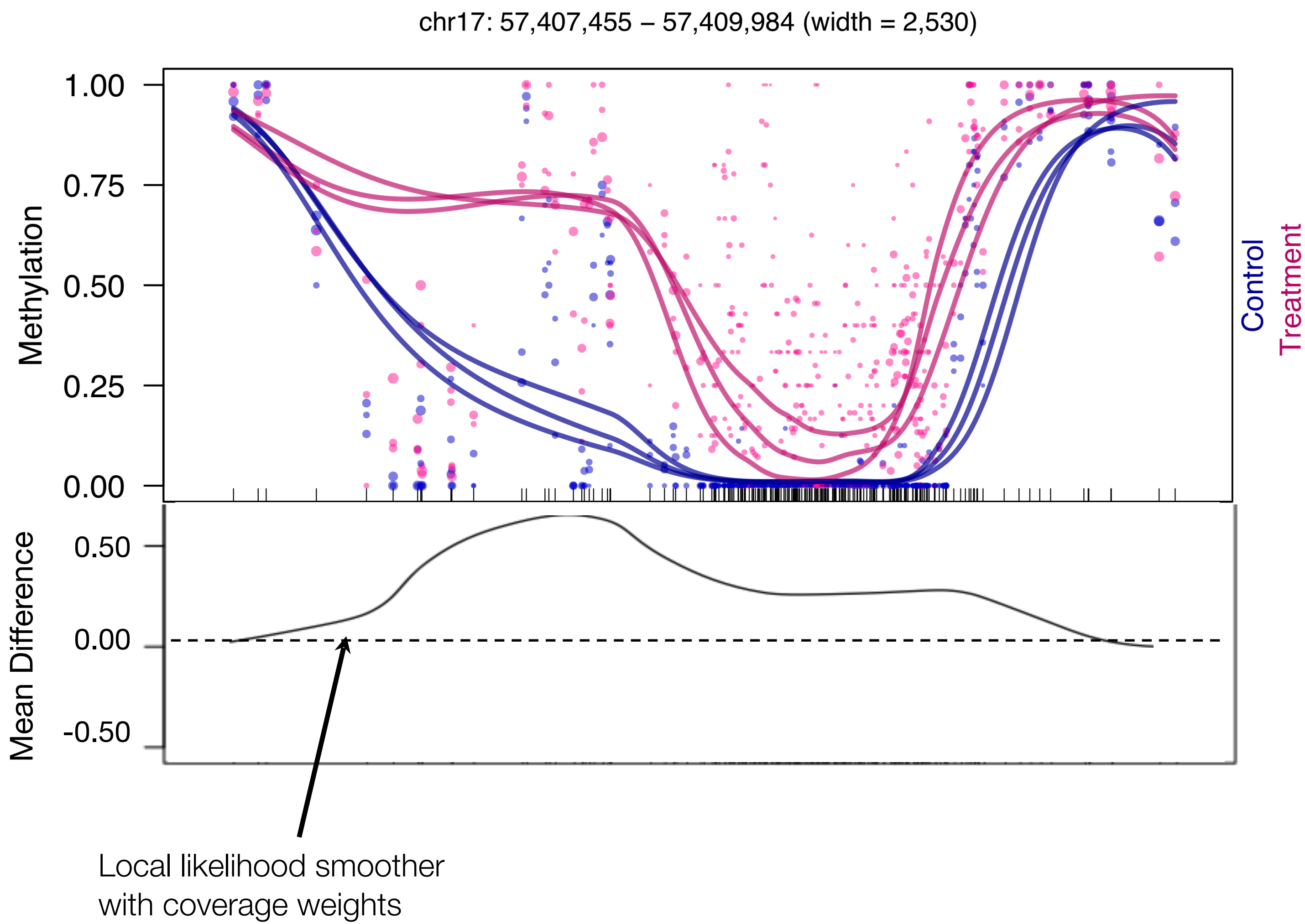

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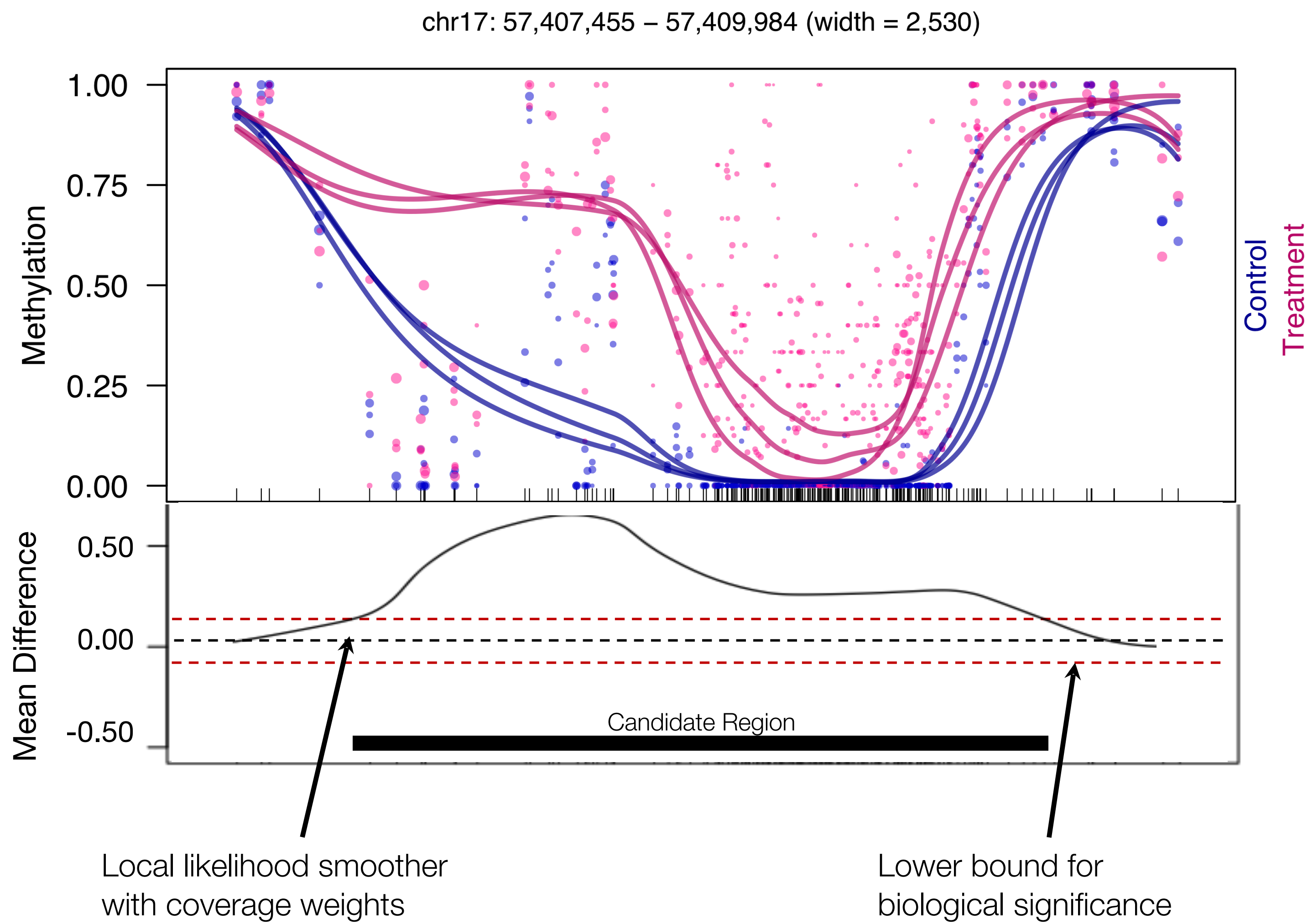
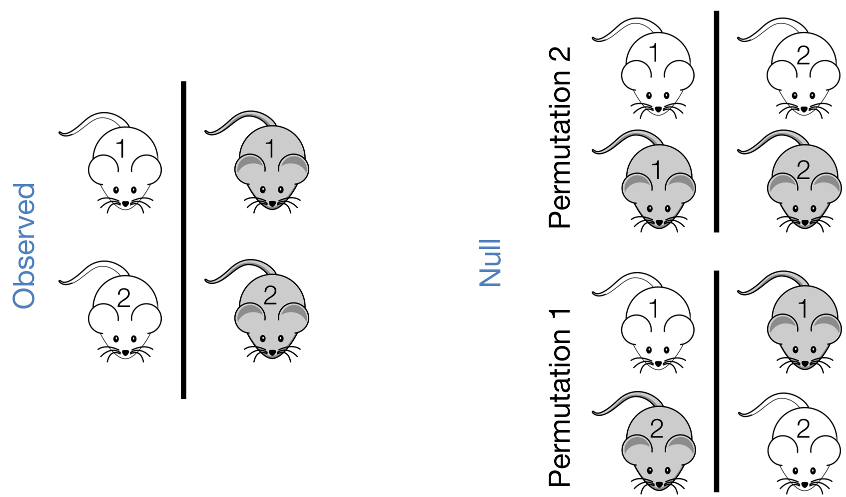


dmrseq: (2) Assess region-level signal

- Formulate region-level summary statistic
- Compare region statistics against null permutation distribution to evaluate significance



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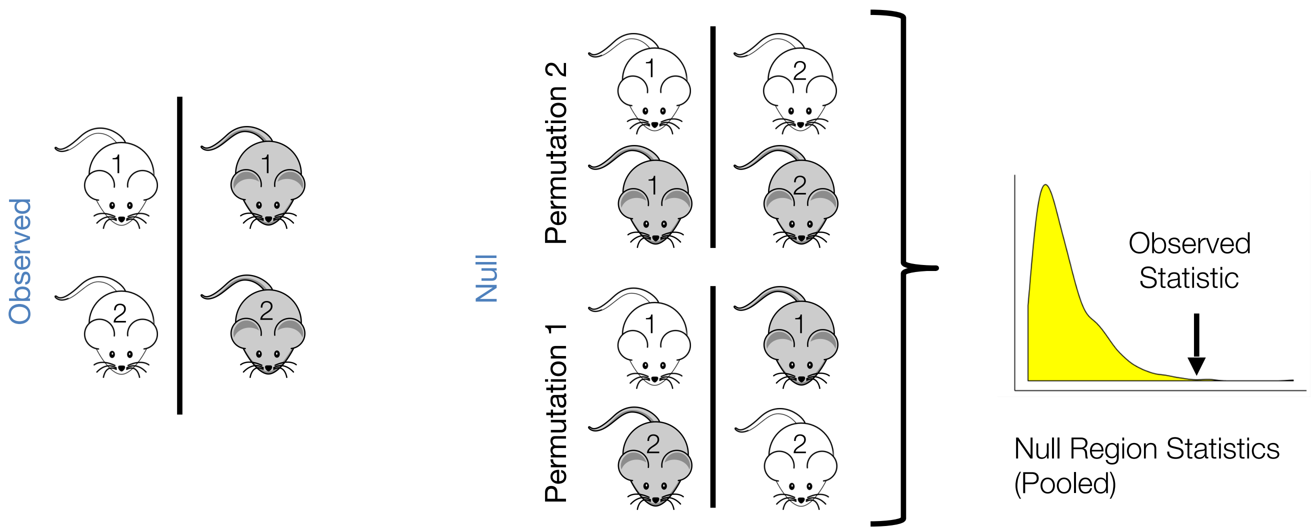
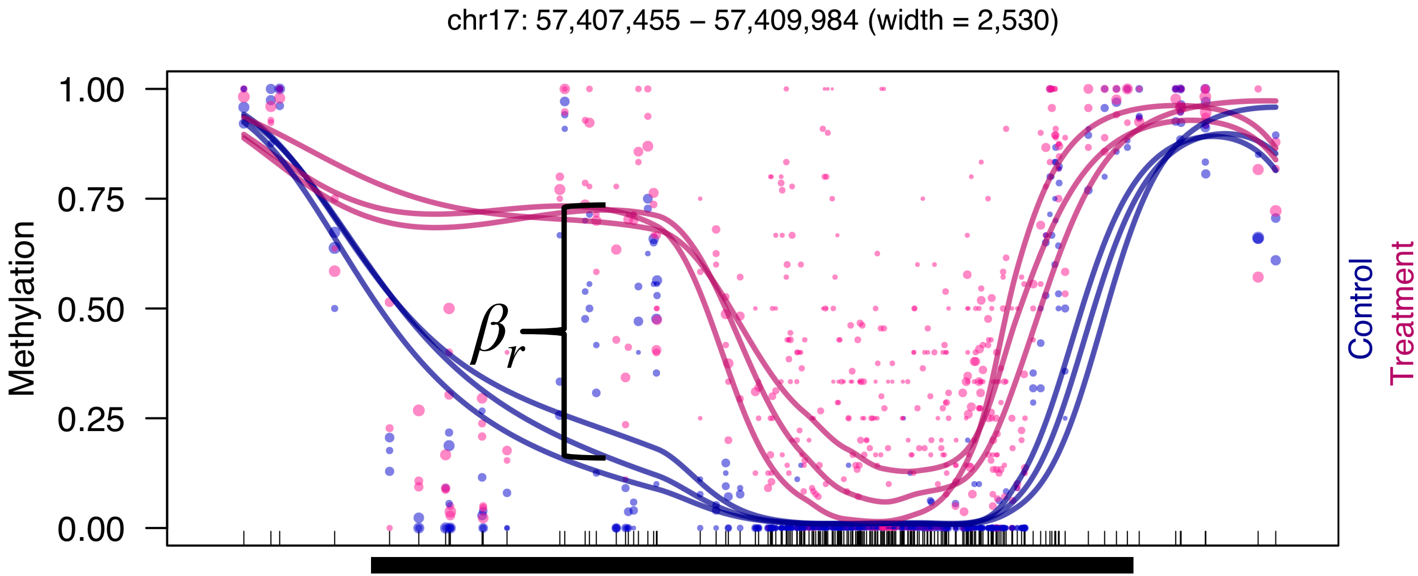


Region-level summary statistic



Captures signal across the entire region, accounting for:

- spatial correlation
- variability within biological condition
- coverage

Evaluation

Simulation to assess FDR and power

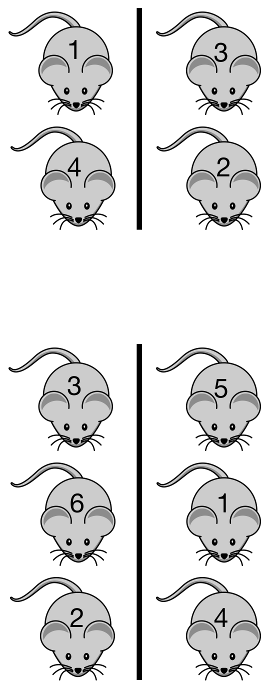
Control samples split into two artificial conditions

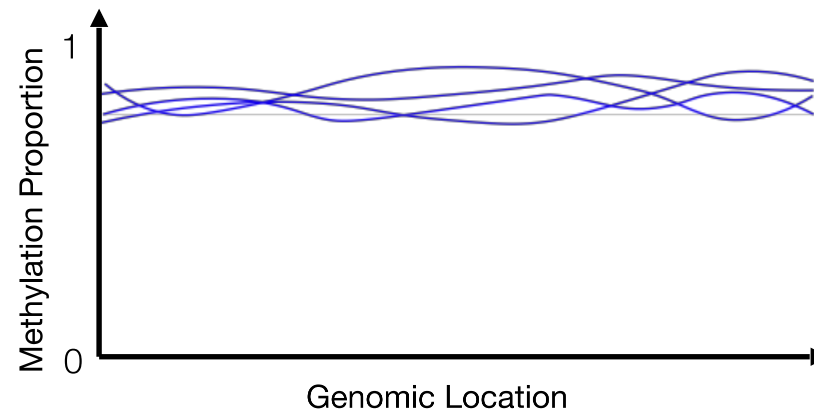
+

In silico DMRs added at random locations

2 vs 2 Comparison

3 vs 3 Comparison





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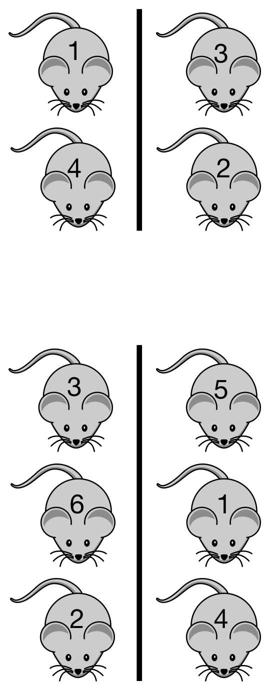
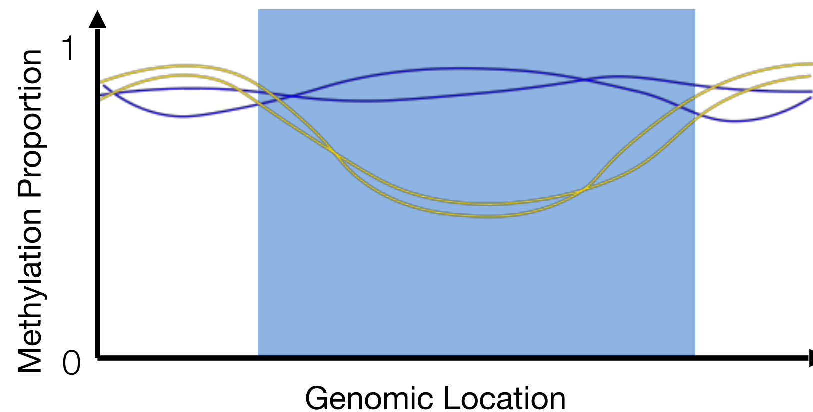
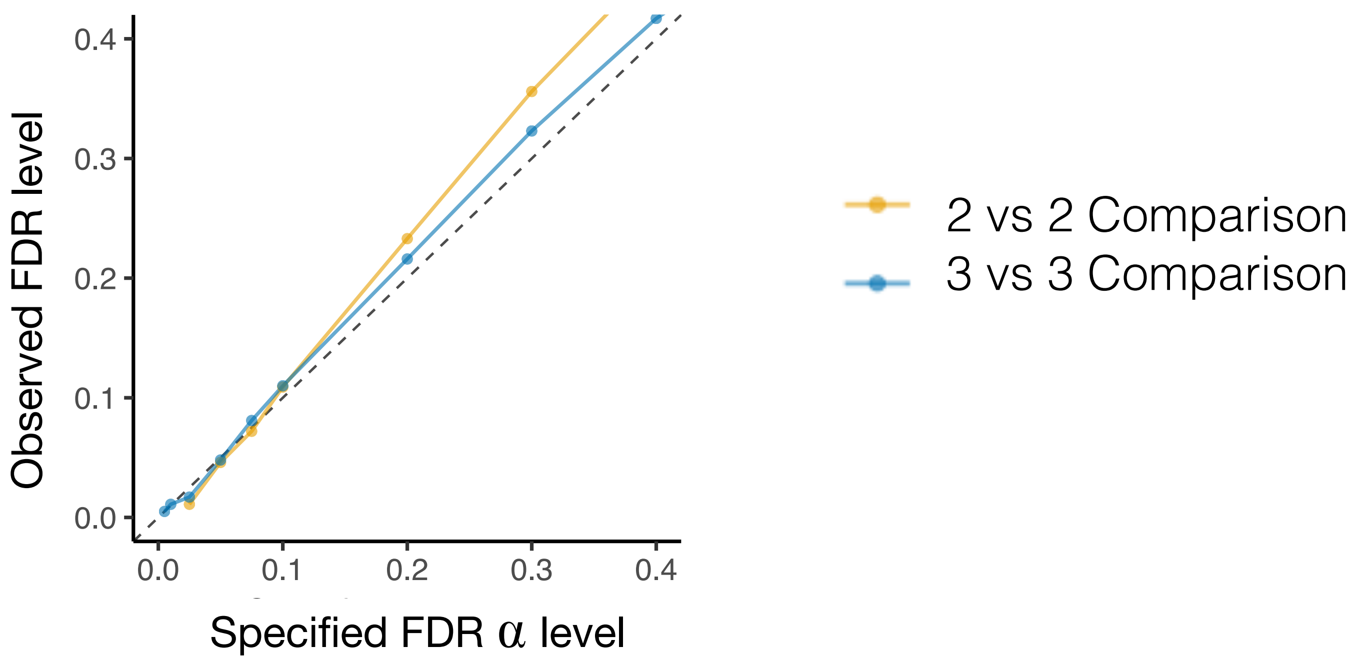


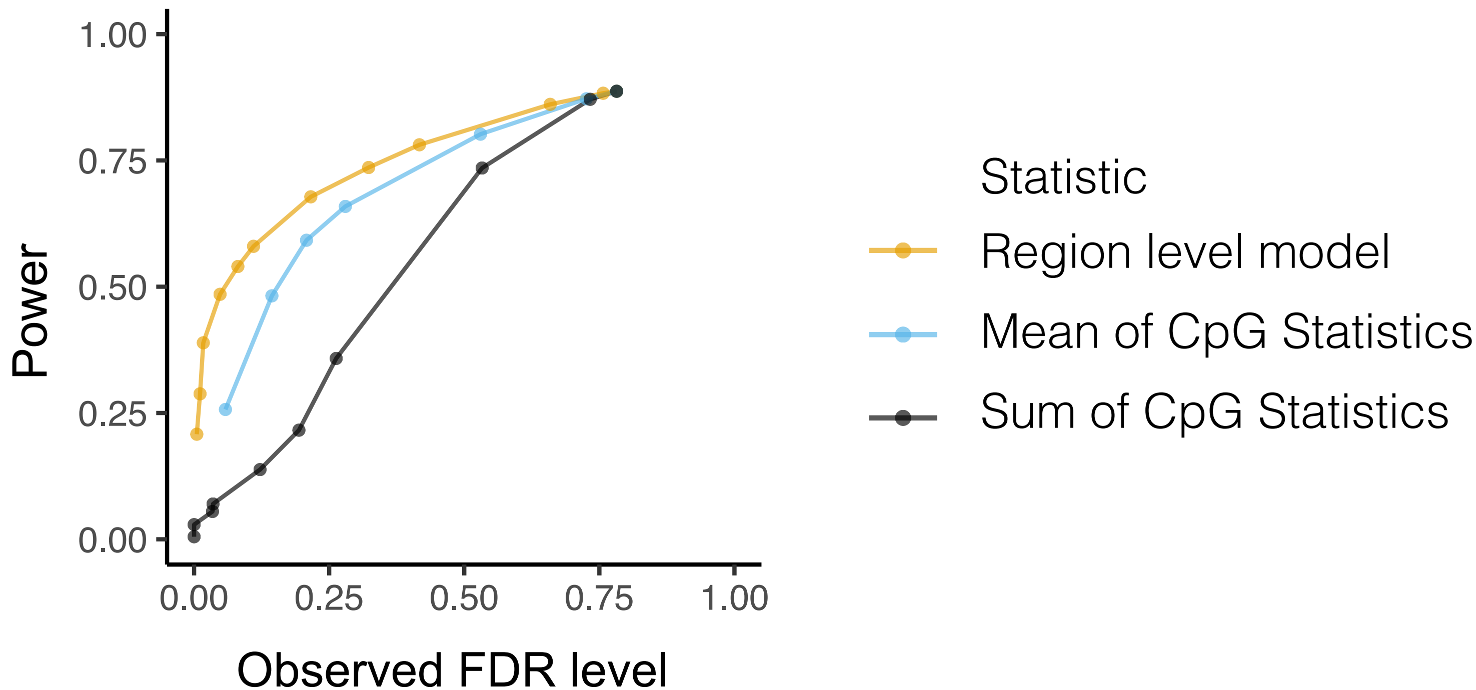


Accurate FDR control in simulation

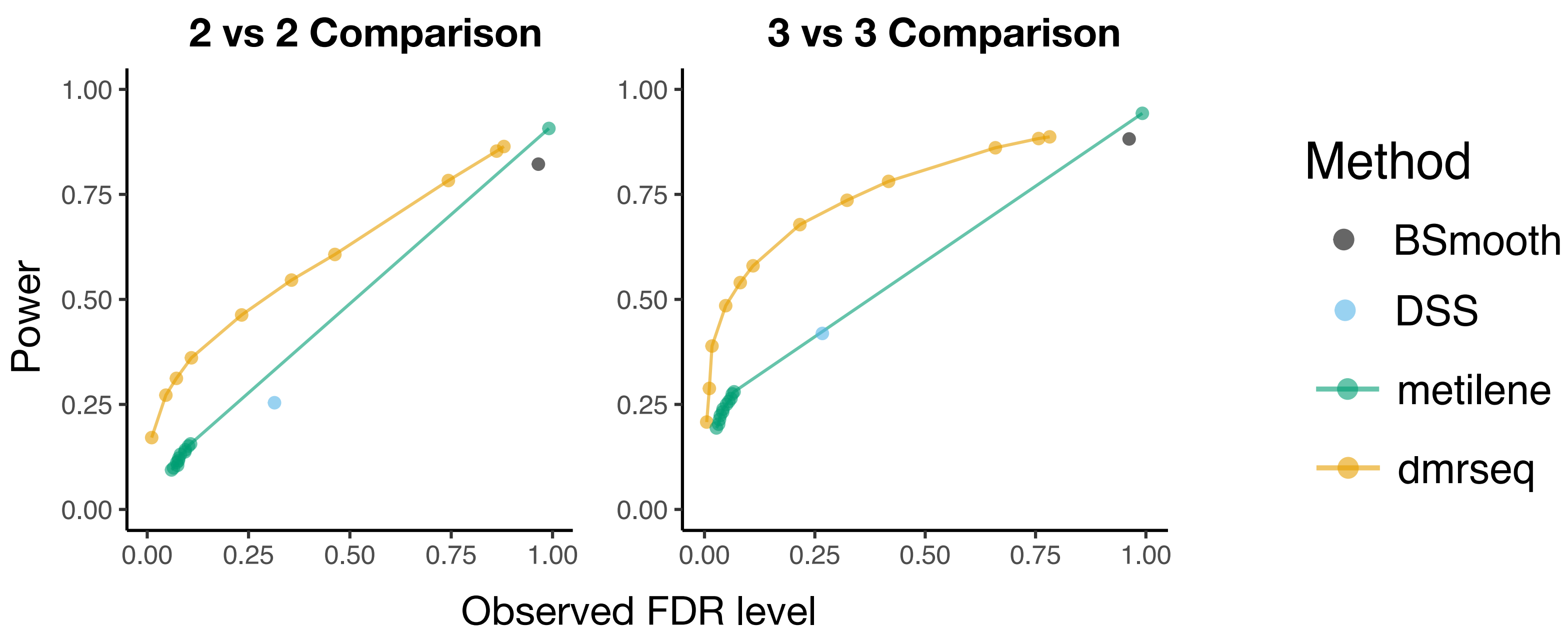


Korthauer et al., 2018 (*Biostatistics*)

Region-level modeling improves power to detect DMRs

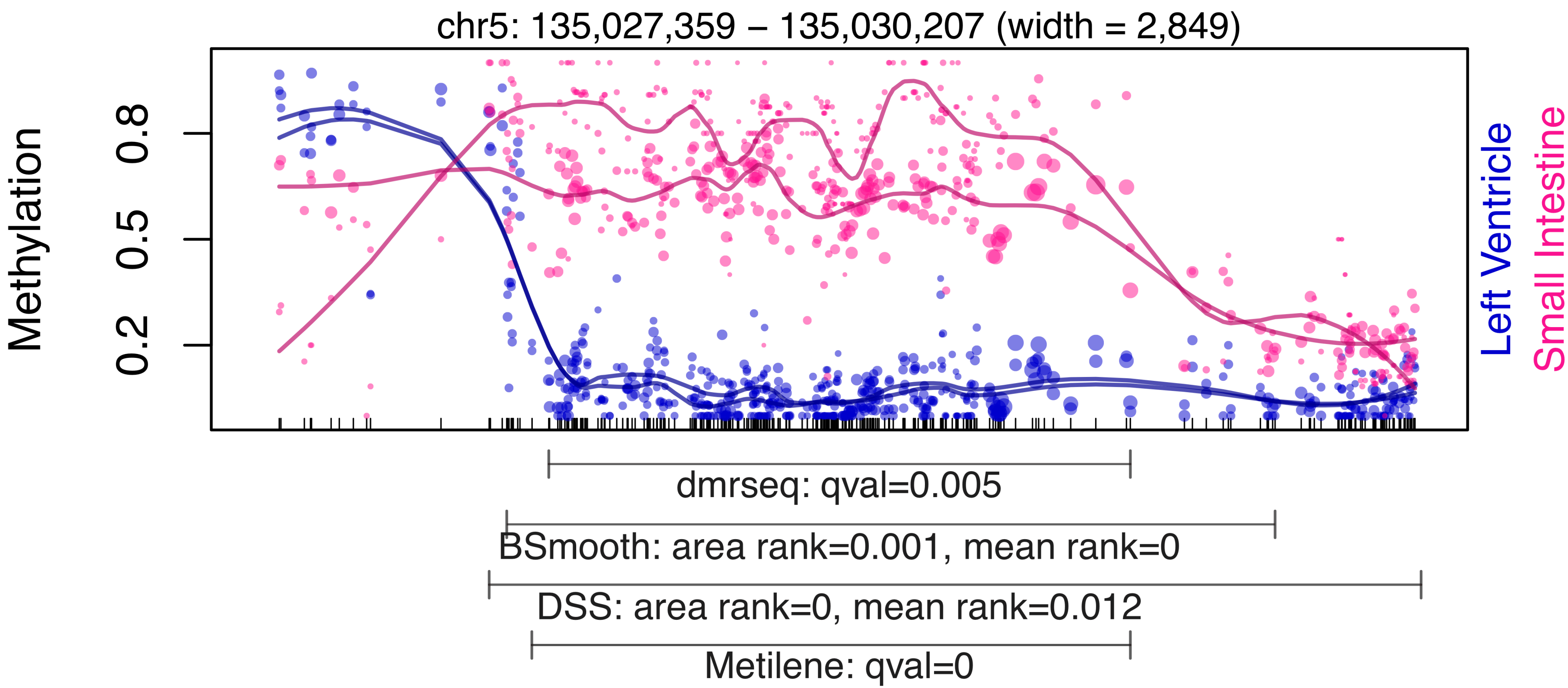


High sensitivity and specificity in simulation



Korthauer et al., 2018 (*Biostatistics*)

Example: highly ranked DMR across all methods



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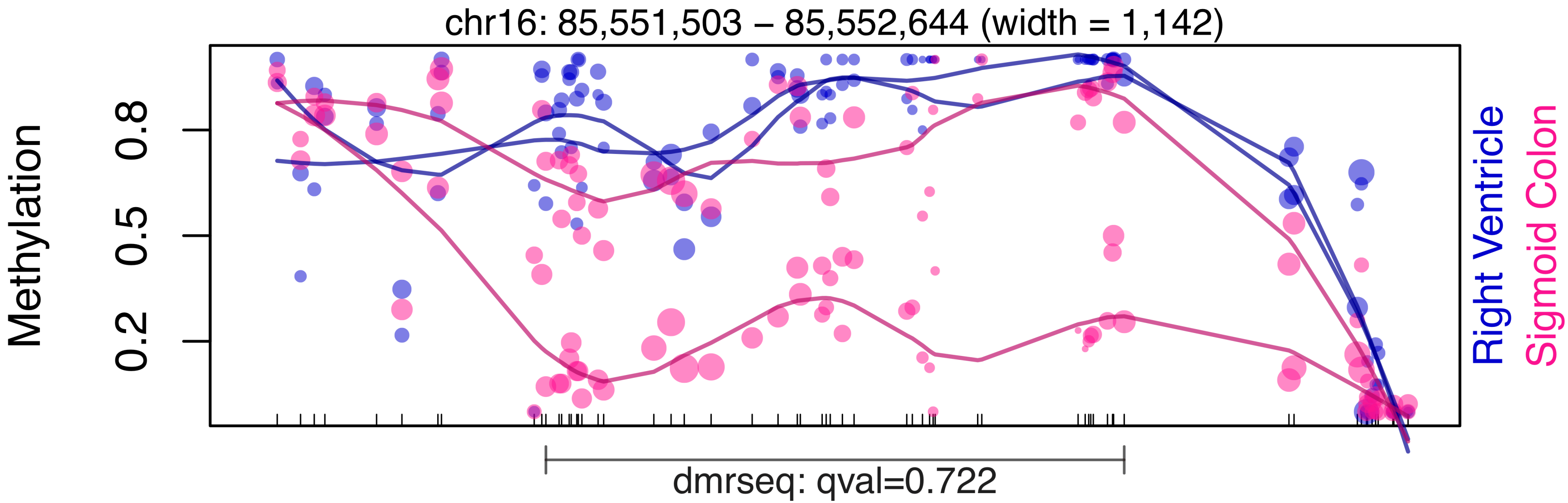
Example: dmrseq accounts for sample variability



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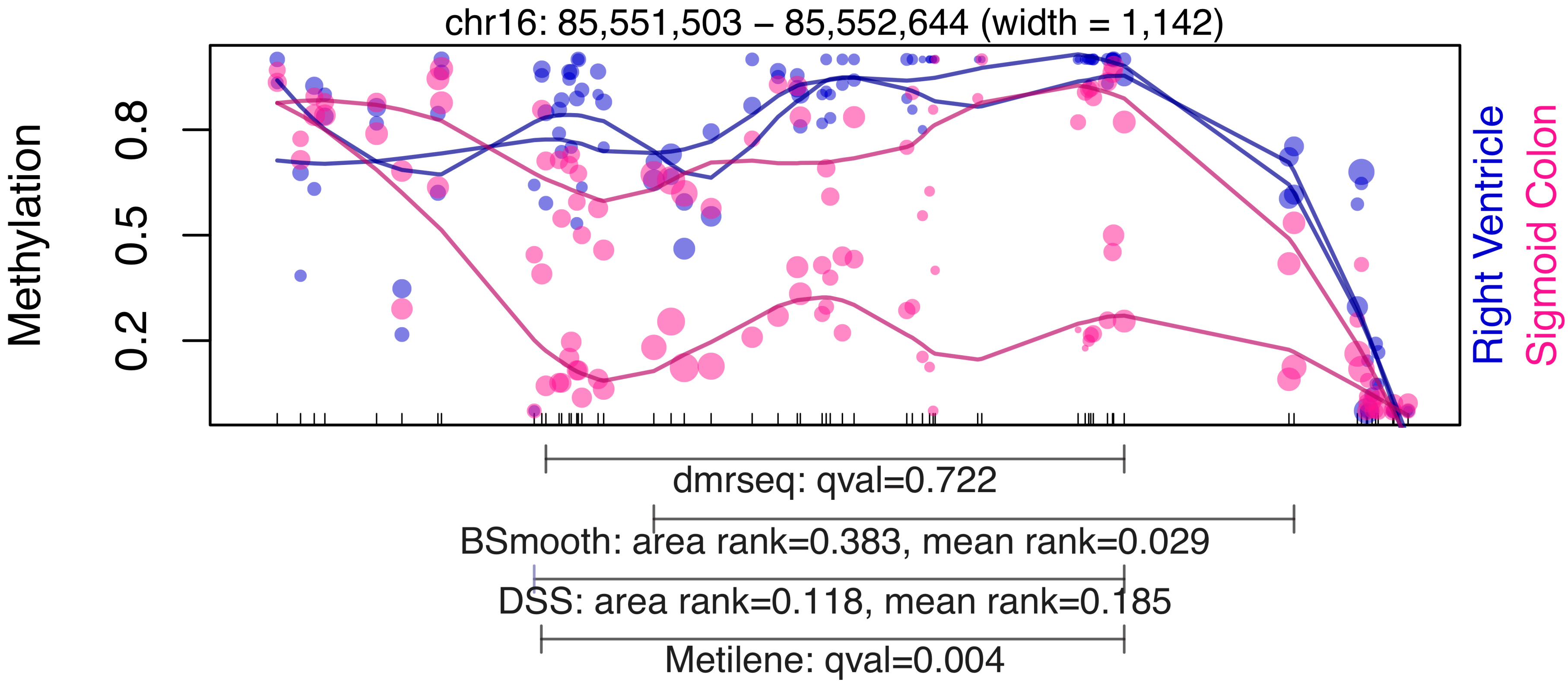

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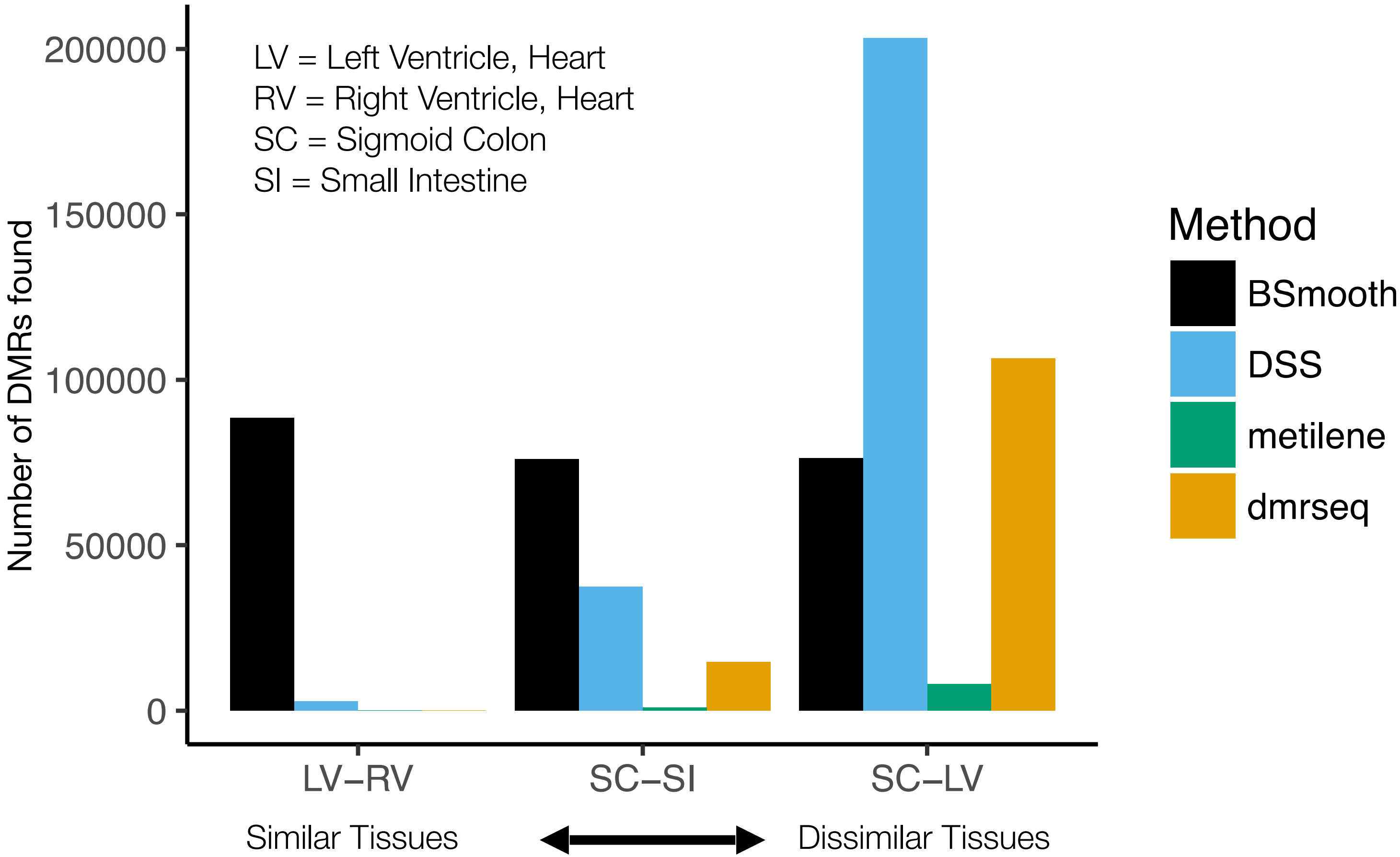
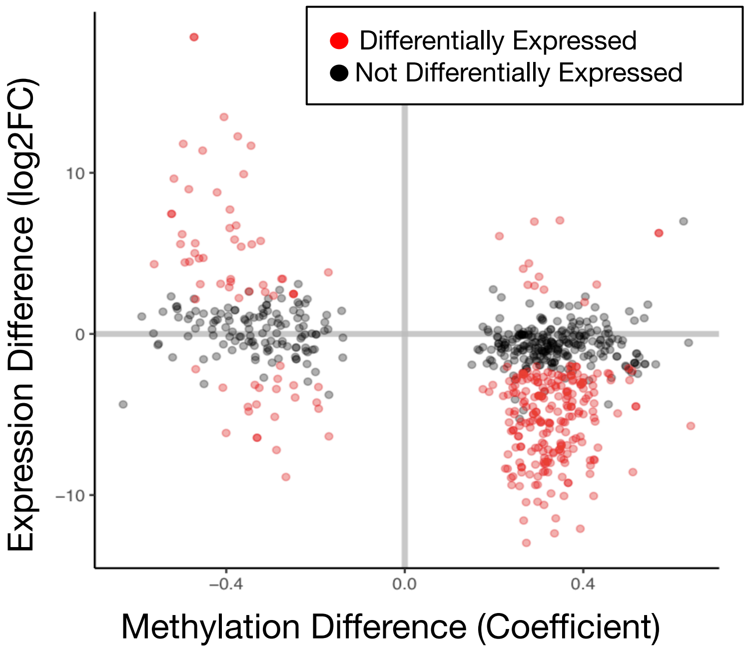
Roadmap case study: Tissue-specific DMRs



Validation of DMRs in promoter regions



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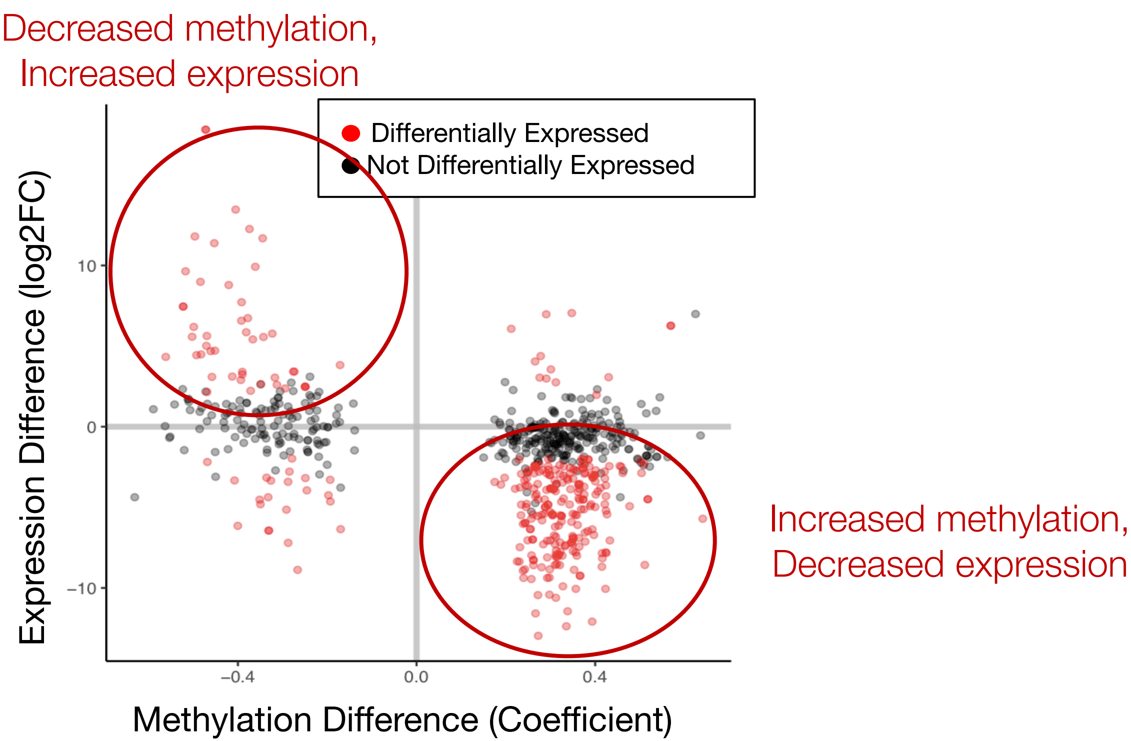

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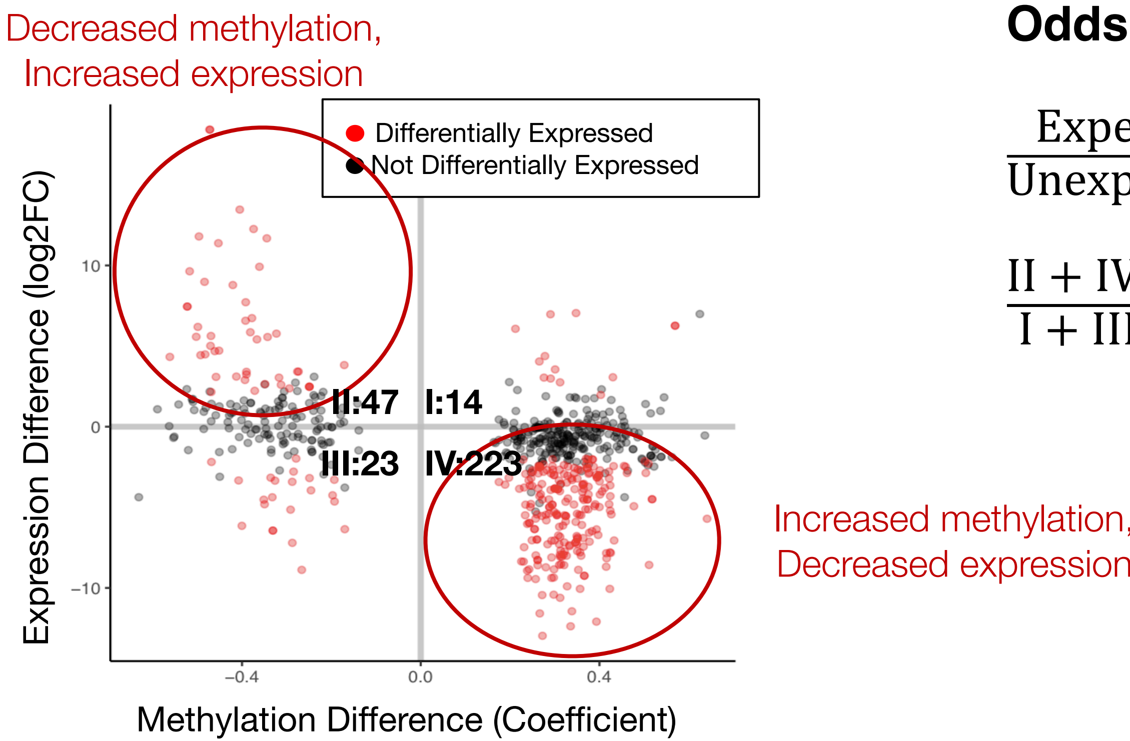


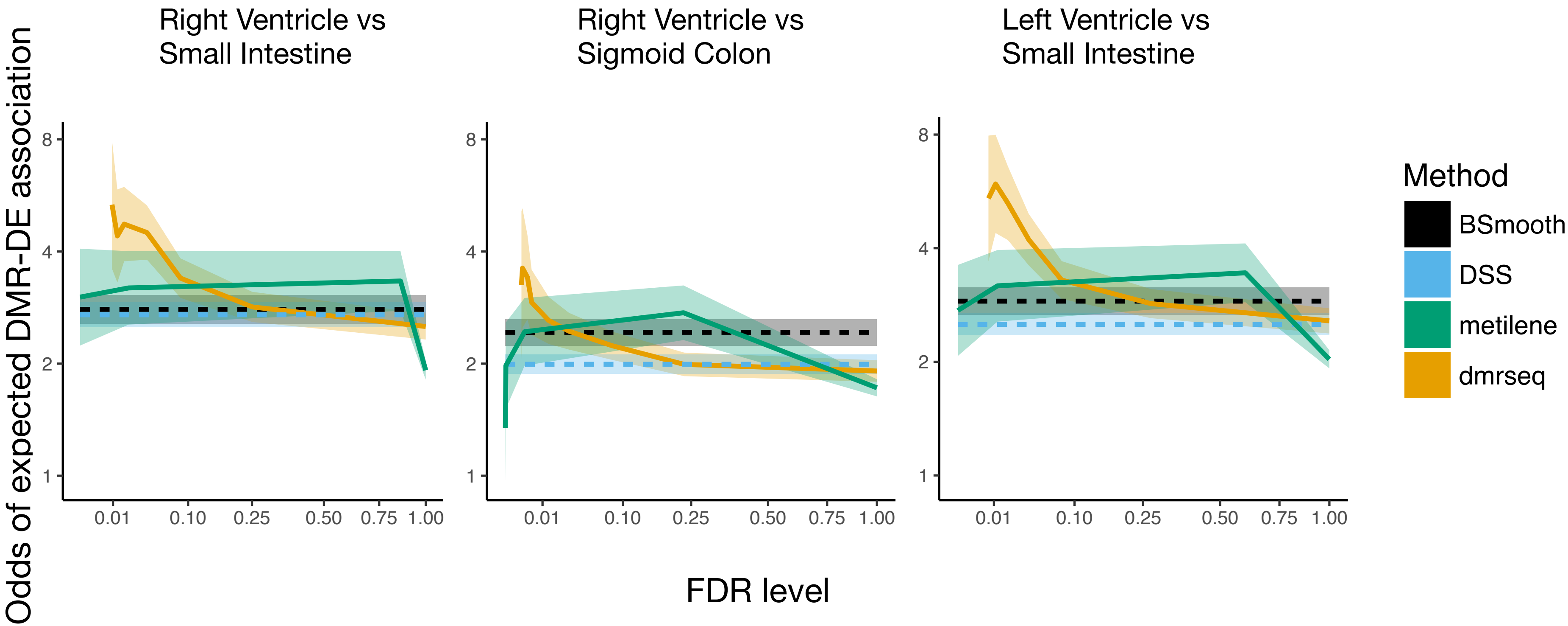
Odds Statistic:

$$\frac{\text{Expected direction}}{\text{Unexpected Direction}} =$$

$$\frac{\mathrm{II} + \mathrm{IV}}{\mathrm{I} + \mathrm{III}} = \frac{47 + 223}{14 + 23} = 7.30$$

Korthauer et al., 2018 (*Biostatistics*)

Validation of DMRs in promoter regions

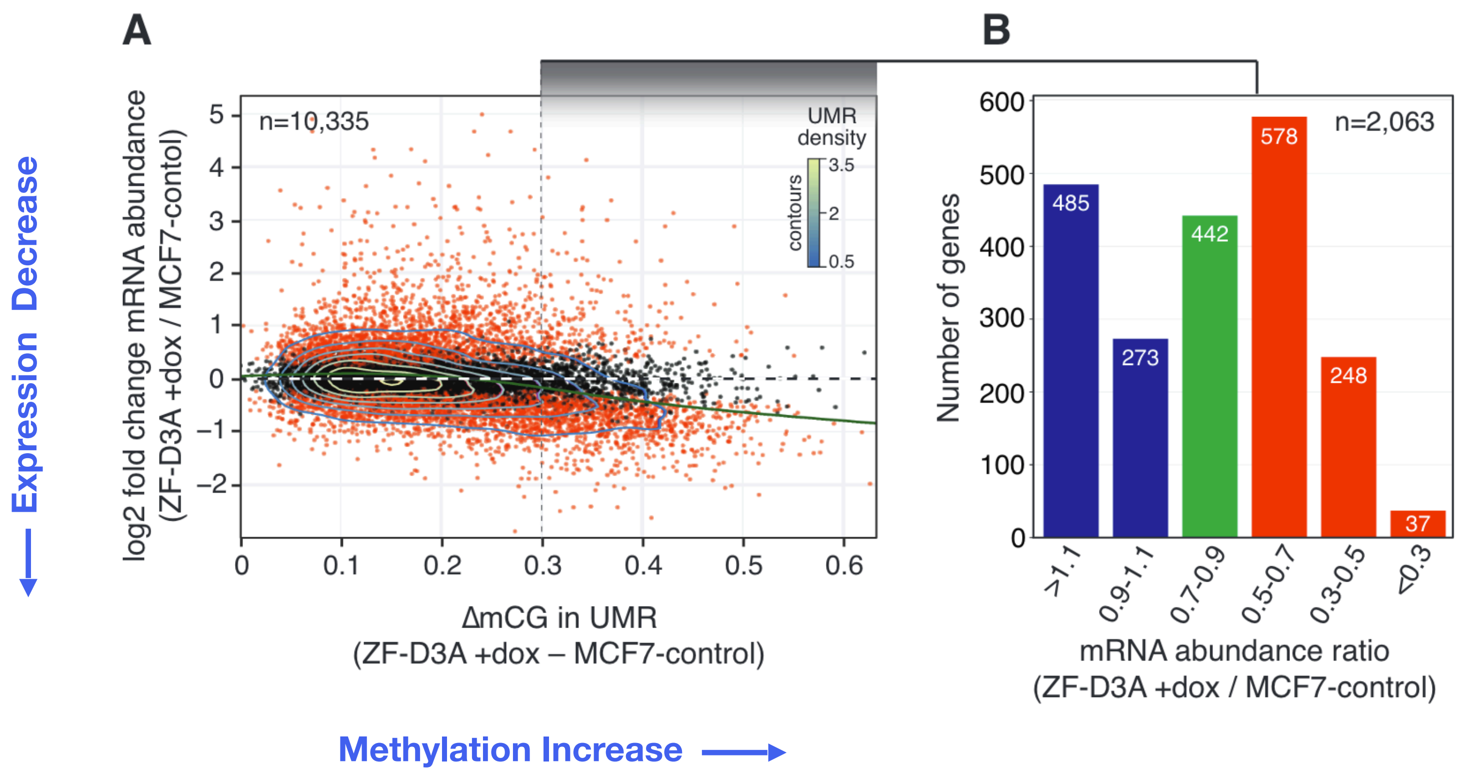


Korthauer et al., 2018 (*Biostatistics*)

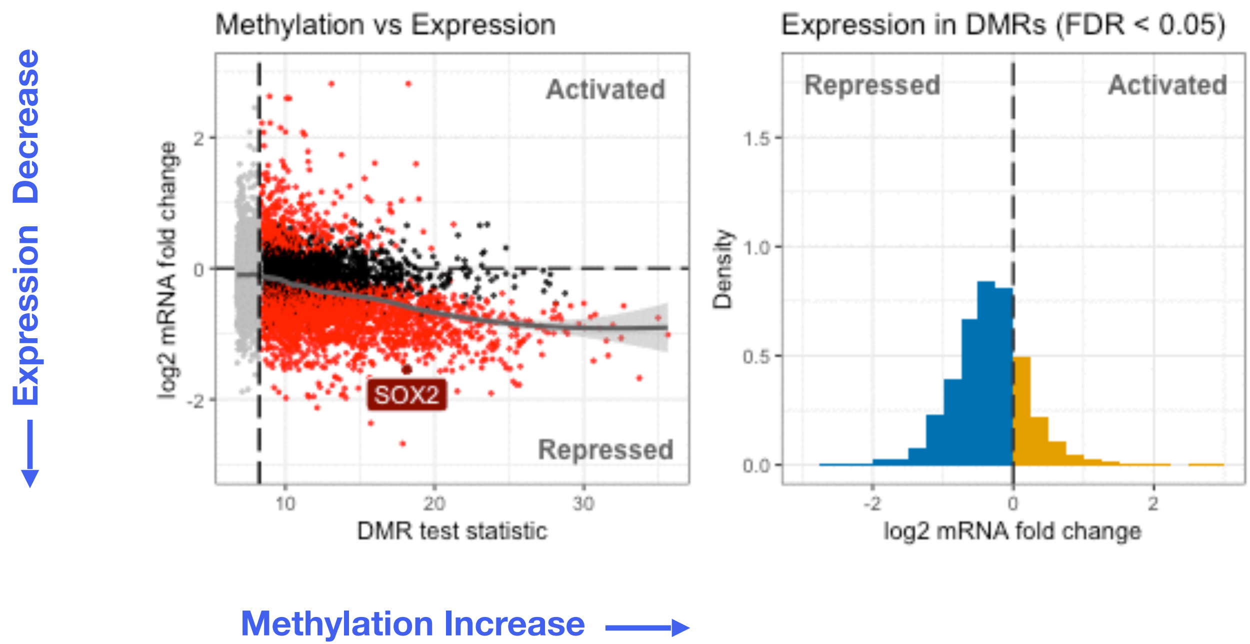
Biological insights

Landmark study finds methylation not generally sufficient to repress gene expression

Figure 5 from Ford et al., 2017 (*bioRxiv*)



Methylation of promoters overwhelmingly represses gene expression



Korthauer & Irizarry, 2018 (*bioRxiv*)

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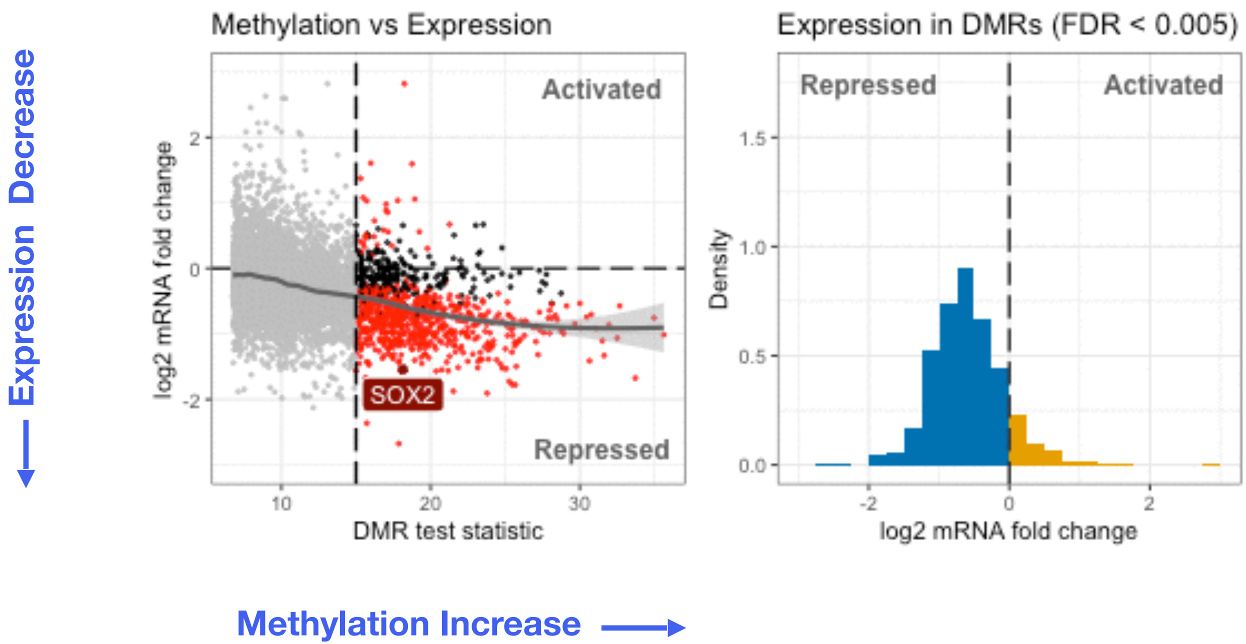

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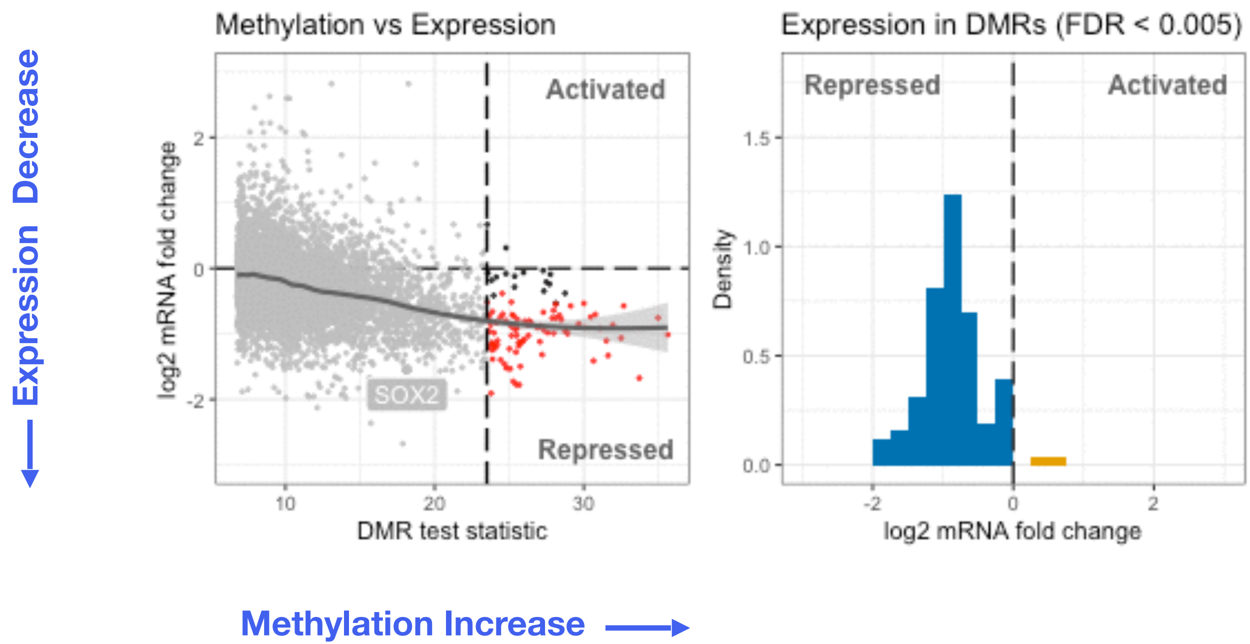
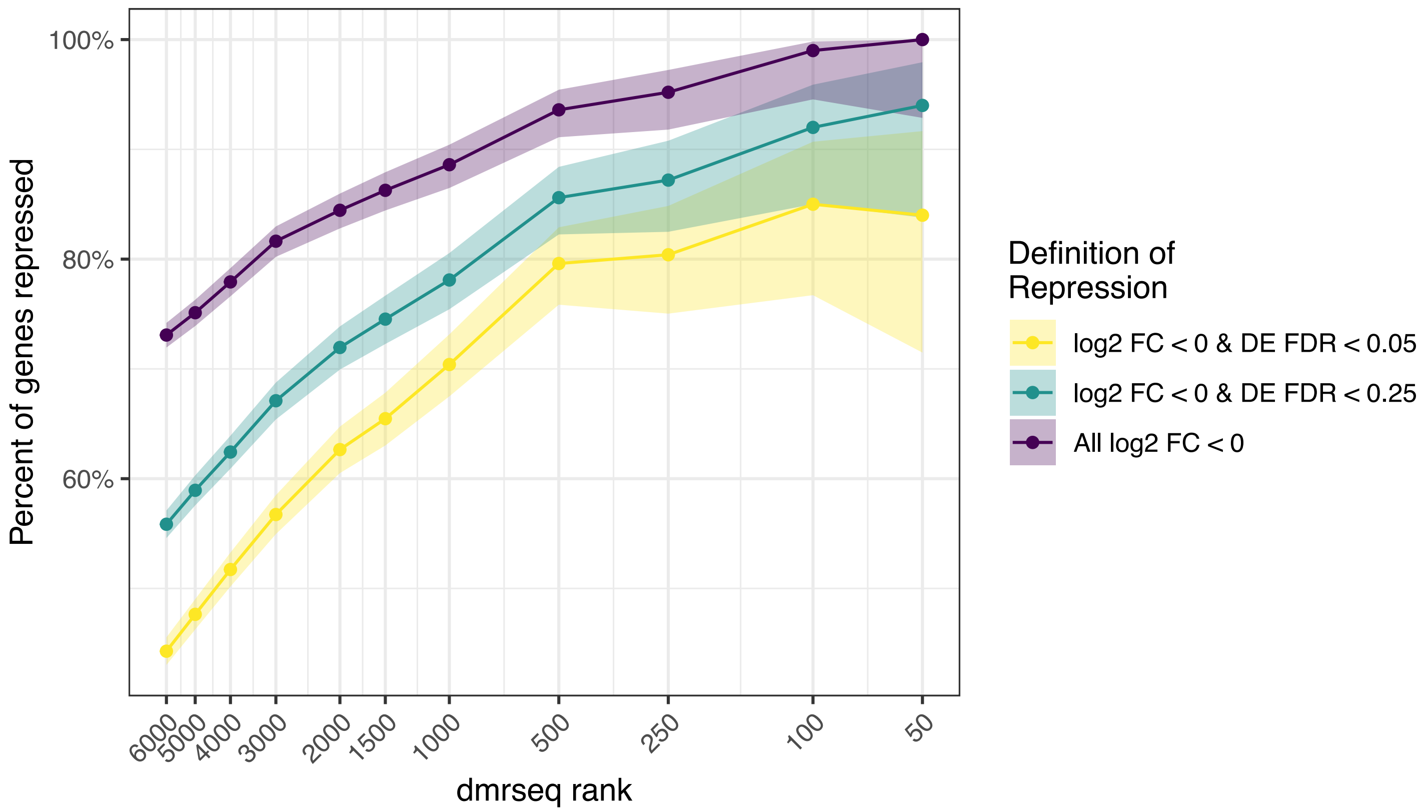


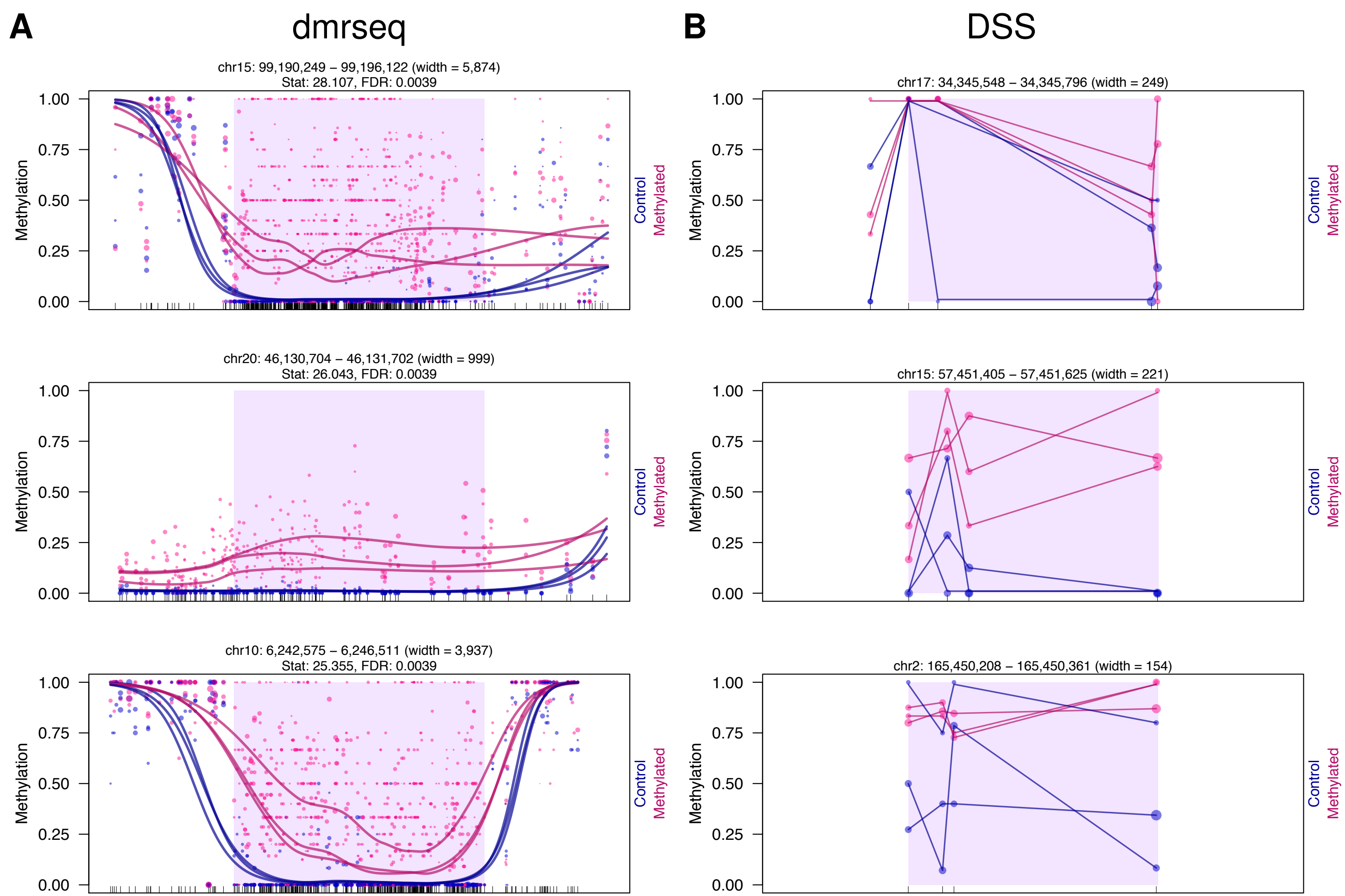
Korthauer & Irizarry, 2018 (*bioRxiv*)

Enrichment increases with significance level



Korthauer & Irizarry, 2018 (*bioRxiv*)

Top-ranked regions found exclusively by each method



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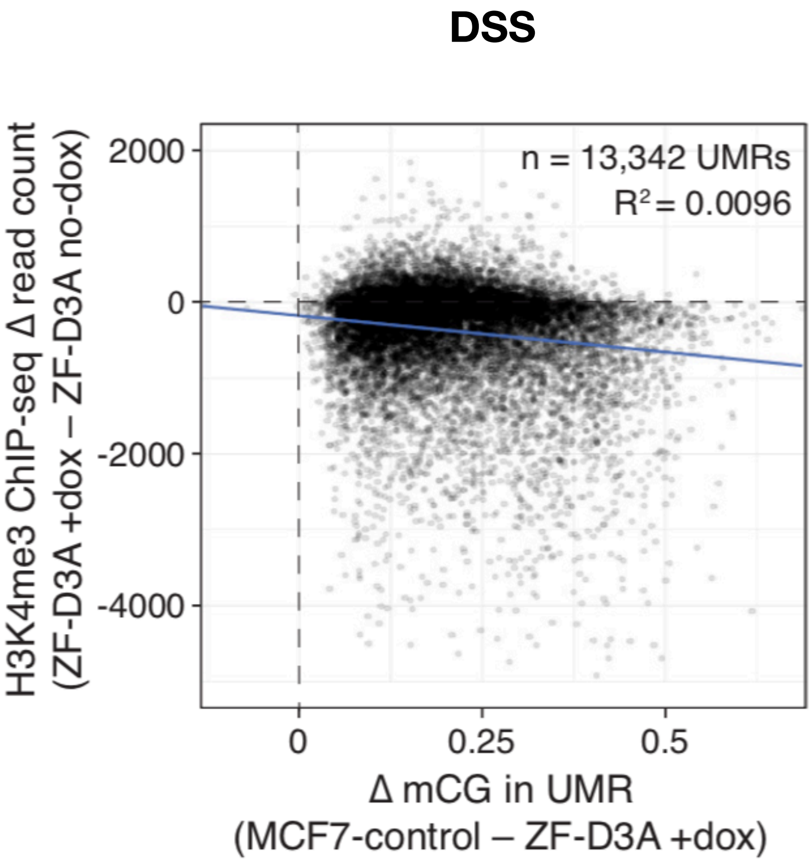
dmrseq shows DNA methylation reduces H3K4 trimethylation



Ford et al., 2017 (*bioRxiv*)

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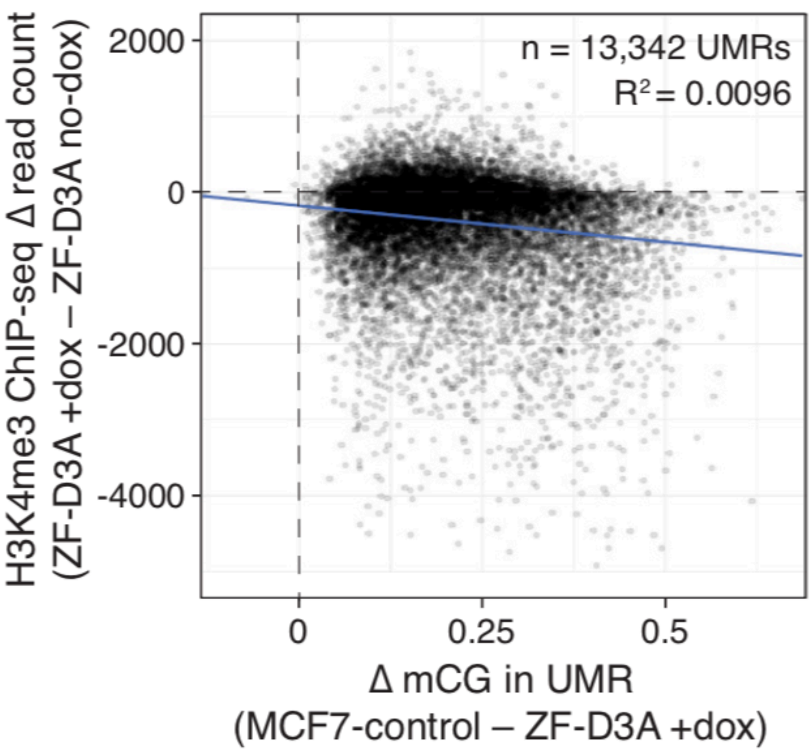
DSS



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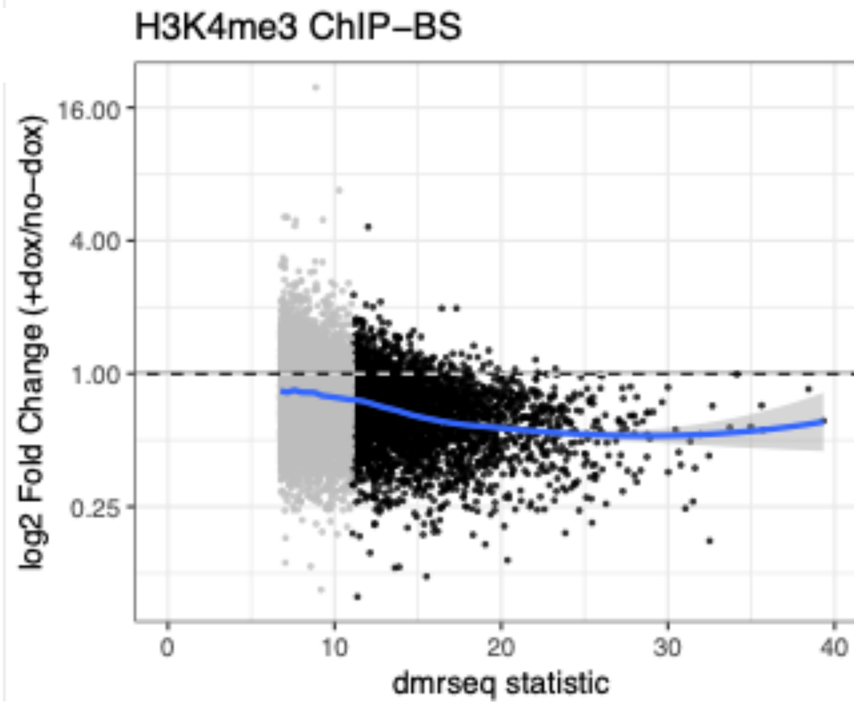
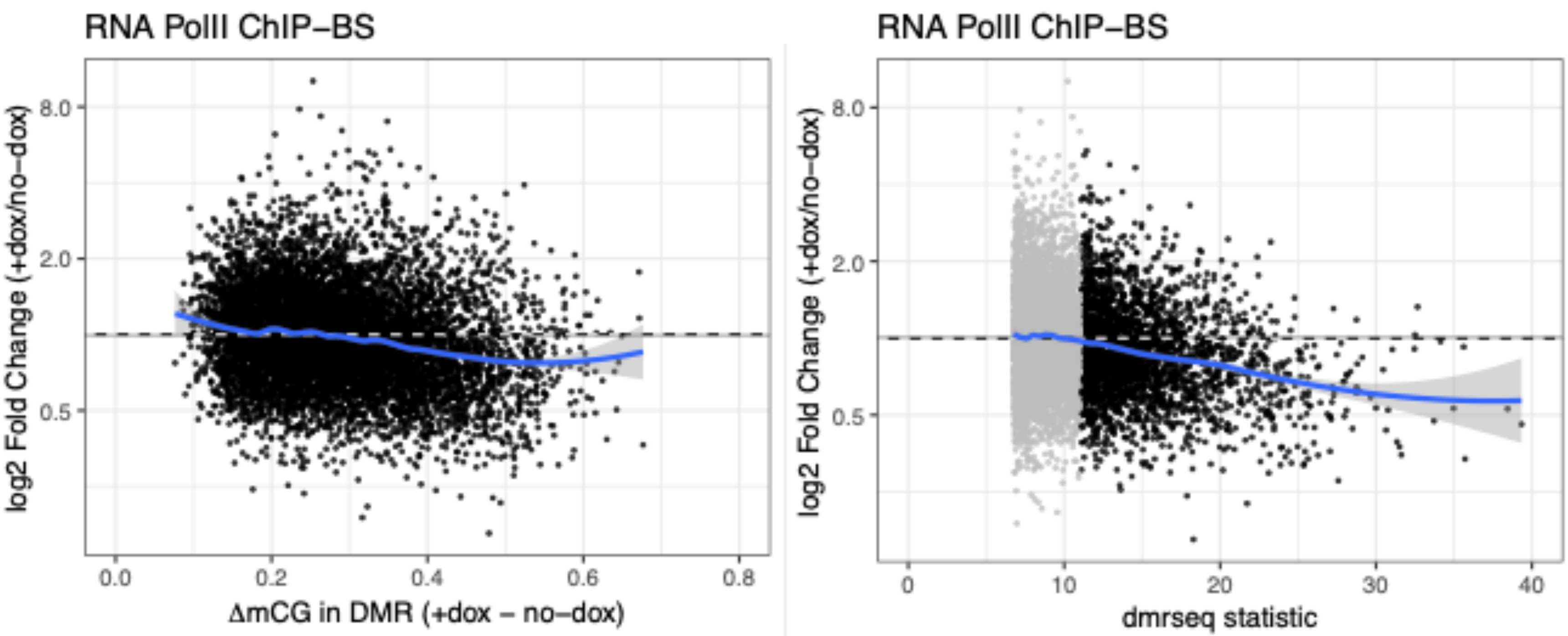
dmrseq



Korthauer & Irizarry, 2018 (*bioRxiv*)

dmrseq shows DNA methylation reduces RNA Pol II activity



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dmrseq R package

dmrseq

platforms all | rank 103 | build ok | updated

DOI: 10.18129/B9.bioc.dm

Detection and infer
Genome Bisulfite S

Bioconductor version: Rele

This package implements a
on differentially methylated
on comparing detected reg
as two samples per populat
least squares (GLS) regress
effect of interest on transfo

Author: Keegan Korthauer
gmail.com>, Yuval Benjam

Maintainer: Keegan Kortha

5 Exploring and exporting results

5.1 Explore how many regions were significant

How many regions were significant at the FDR (q-value) cutoff of 0.05? We can find this by counting how many values in the `qval` column of the results data.frame were less than 0.05. You can also subset the regions by an FDR cutoff.

```
sum(regions$qval < 0.05)
```

```
## [1] 144
```

```
# select just the regions below FDR 0.05 and place in a new data.frame
sigRegions <- regions[regions$qval < 0.05,]
```

5.2 Hypo- or Hyper- methylation?

You can determine the proportion of regions with hyper-methylation by counting how many had a positive direction of effect (positive statistic).

```
sum(sigRegions$stat > 0) / length(sigRegions)
```

```
## [1] 0.25
```

To interpret the direction of effect, note that for a two-group comparison **dmrseq** uses alphabetical order of the covariate of interest. The condition with a higher alphabetical rank will become the reference category. For example, if the two conditions are "A" and "B", the "A" group will be the reference category, so a positive direction of effect means that "B" is hyper-methylated relative to "A". Conversely, a negative direction of effect means that "B" is hypo-methylated relative to "A".

5.3 Plot DMRs

diff
2
4

Summary

- dmrseq **identifies and prioritizes DMRs** from bisulfite sequencing experiments
    - **Models region level methylation differences** in order to account for sample and spatial variability
    - Quantifies uncertainty using permutation in order to achieve **accurate false discovery rate control**
    - **Reveals the expected link between DNA methylation and gene expression** in the reanalysis of a landmark study
- R package implementation:
- Reproducible analyses from Korthauer et al. (2018, *Biostatistics*) and Korthauer & Irizarry (2018, *bioRxiv*):

Acknowledgements

Harvard Biostatistics & DFCI Data Sciences

Rafael Irizarry
Claire Duvallet
Stephanie Hicks
Patrick Kimes
Yered Pita-Juarez
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