Selected Topics in ChIP-seq data analysis

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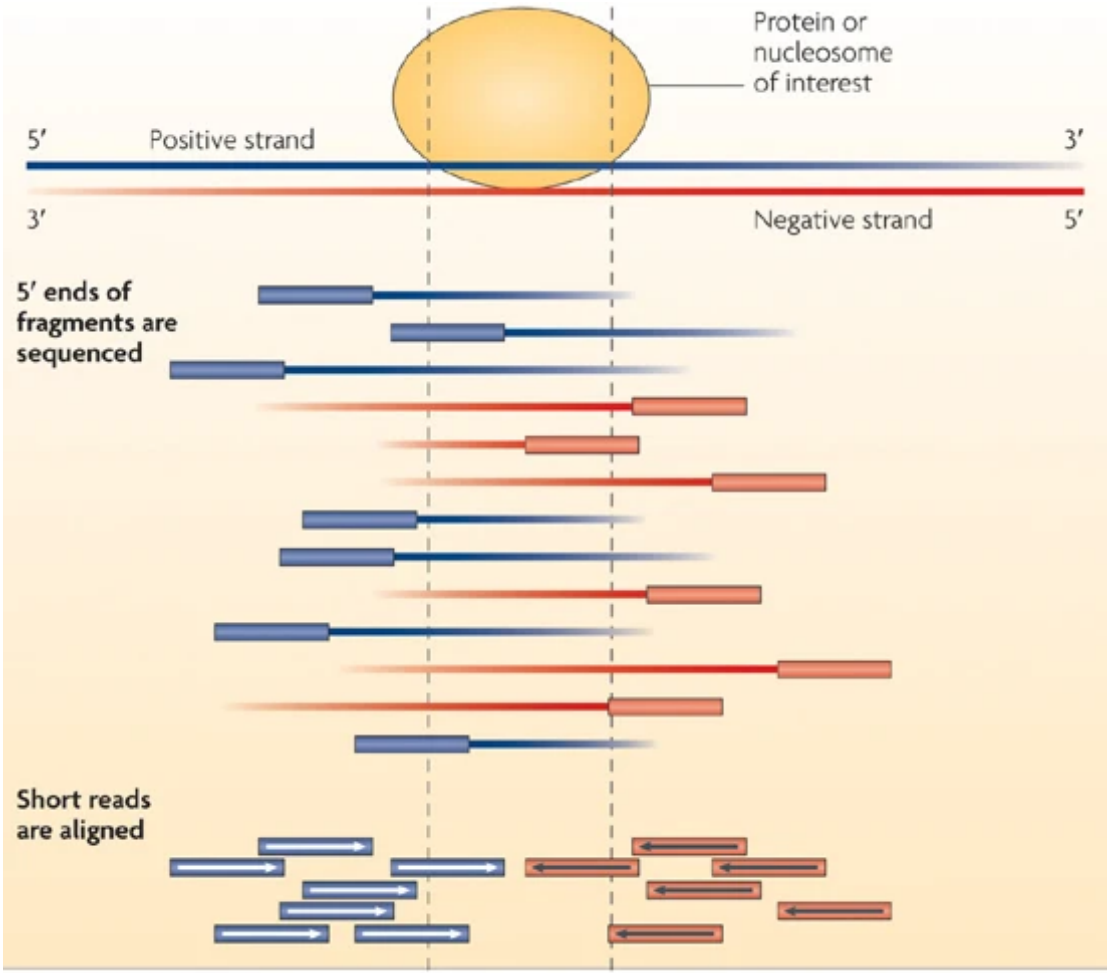
03.03.2021

Overview

- Introduction
- ChIP-seq Coverage
- Normalization Methods
- Peak Overlaps
- Statistical Analysis

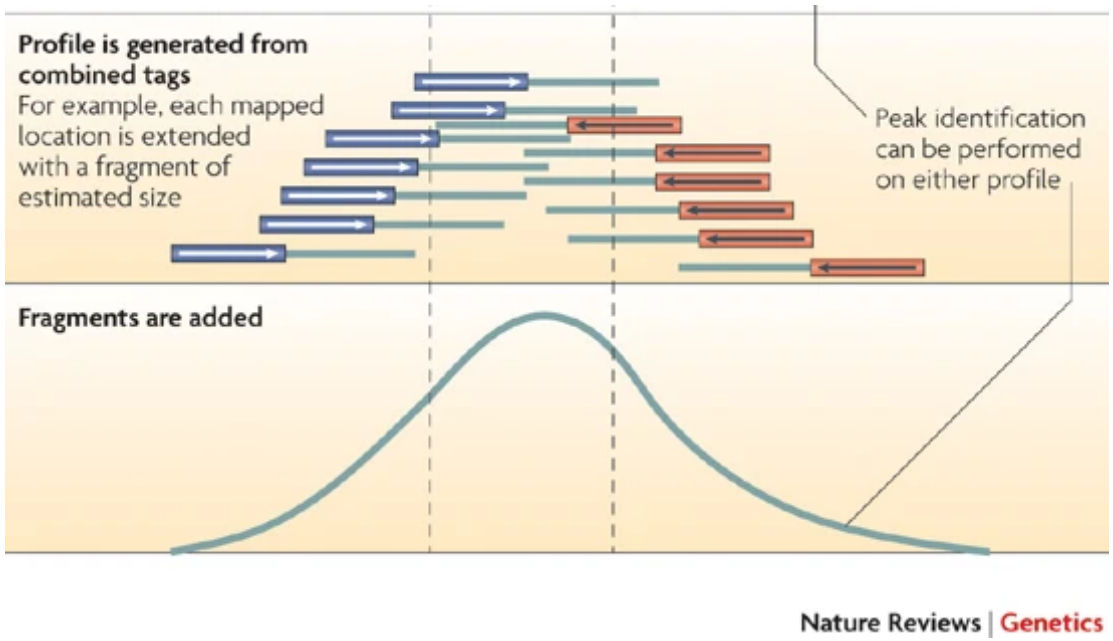
Source: https://github.com/tschauer/ChIPseq_Talk

Introduction



Peter J. Park, 2009

Introduction



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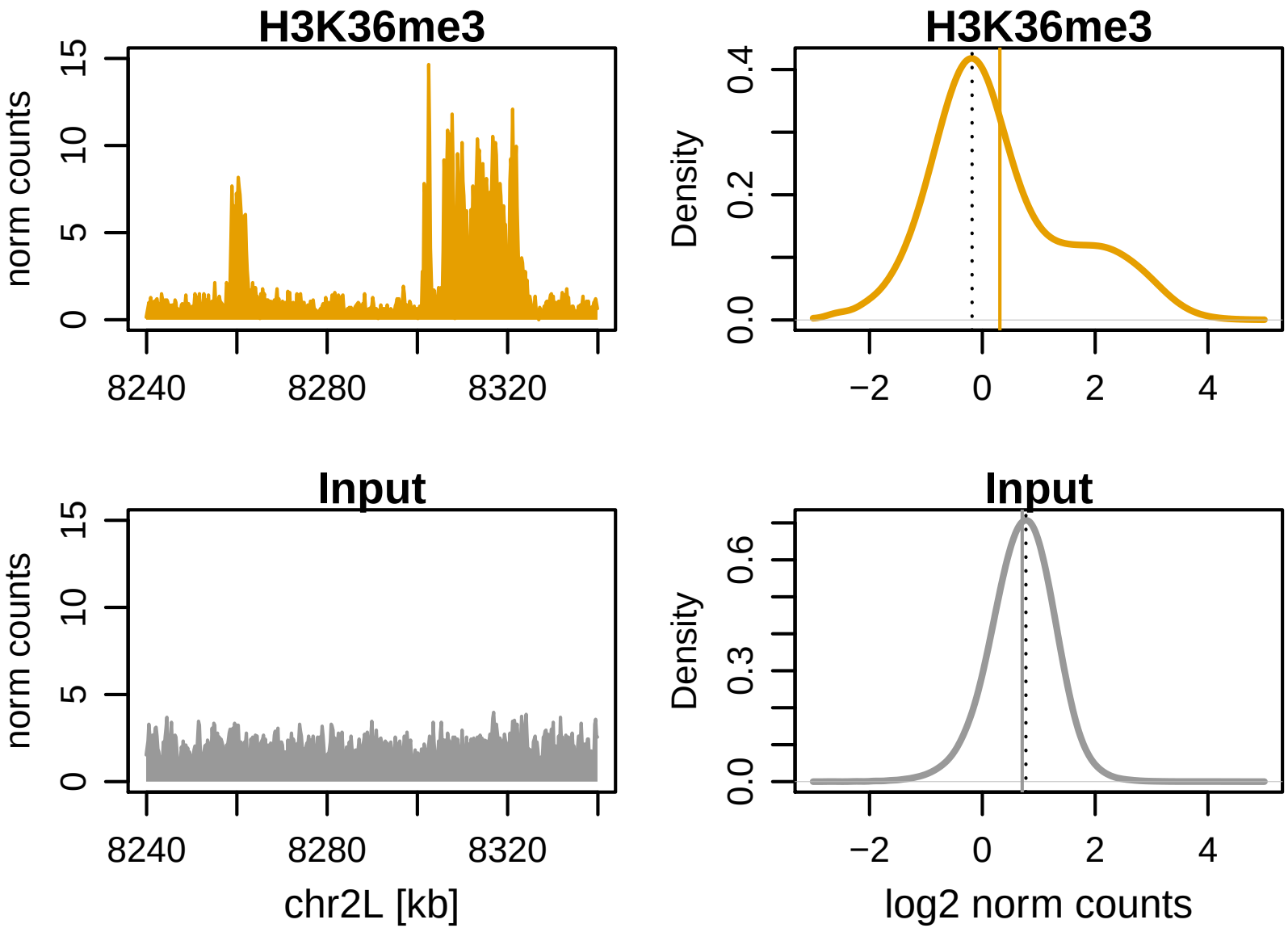
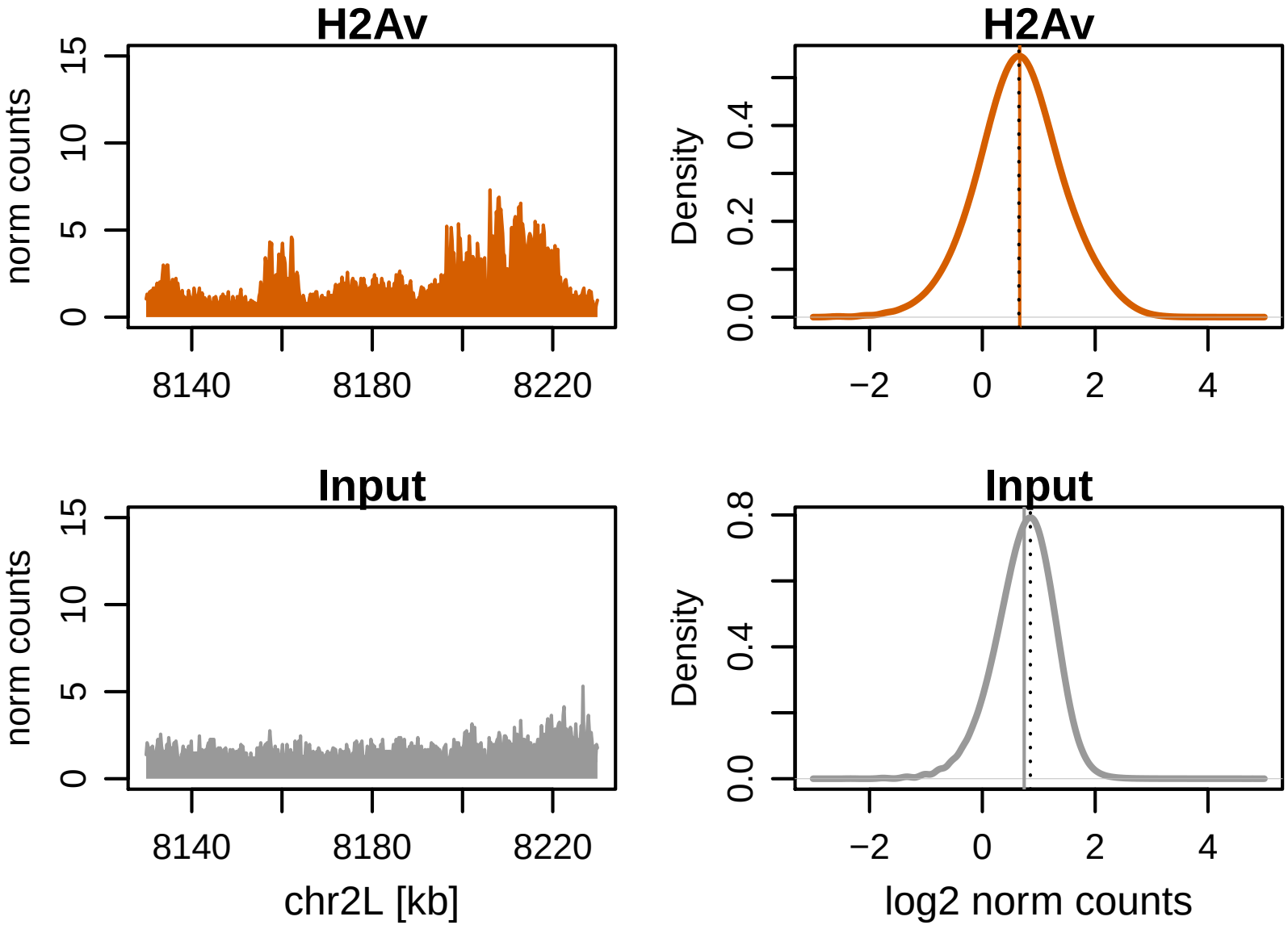
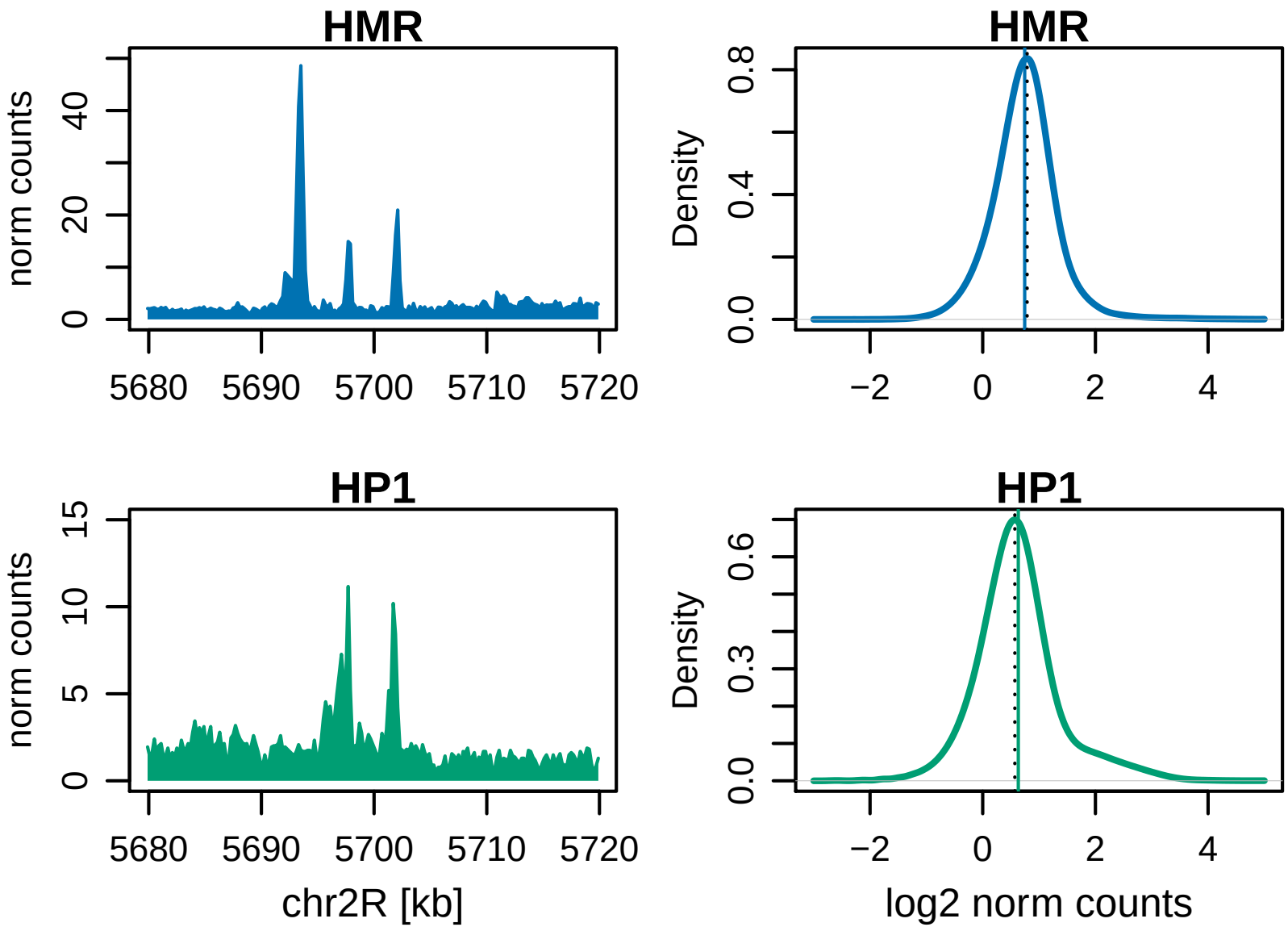
ChIP Coverage



ChIP Coverage



ChIP Coverage



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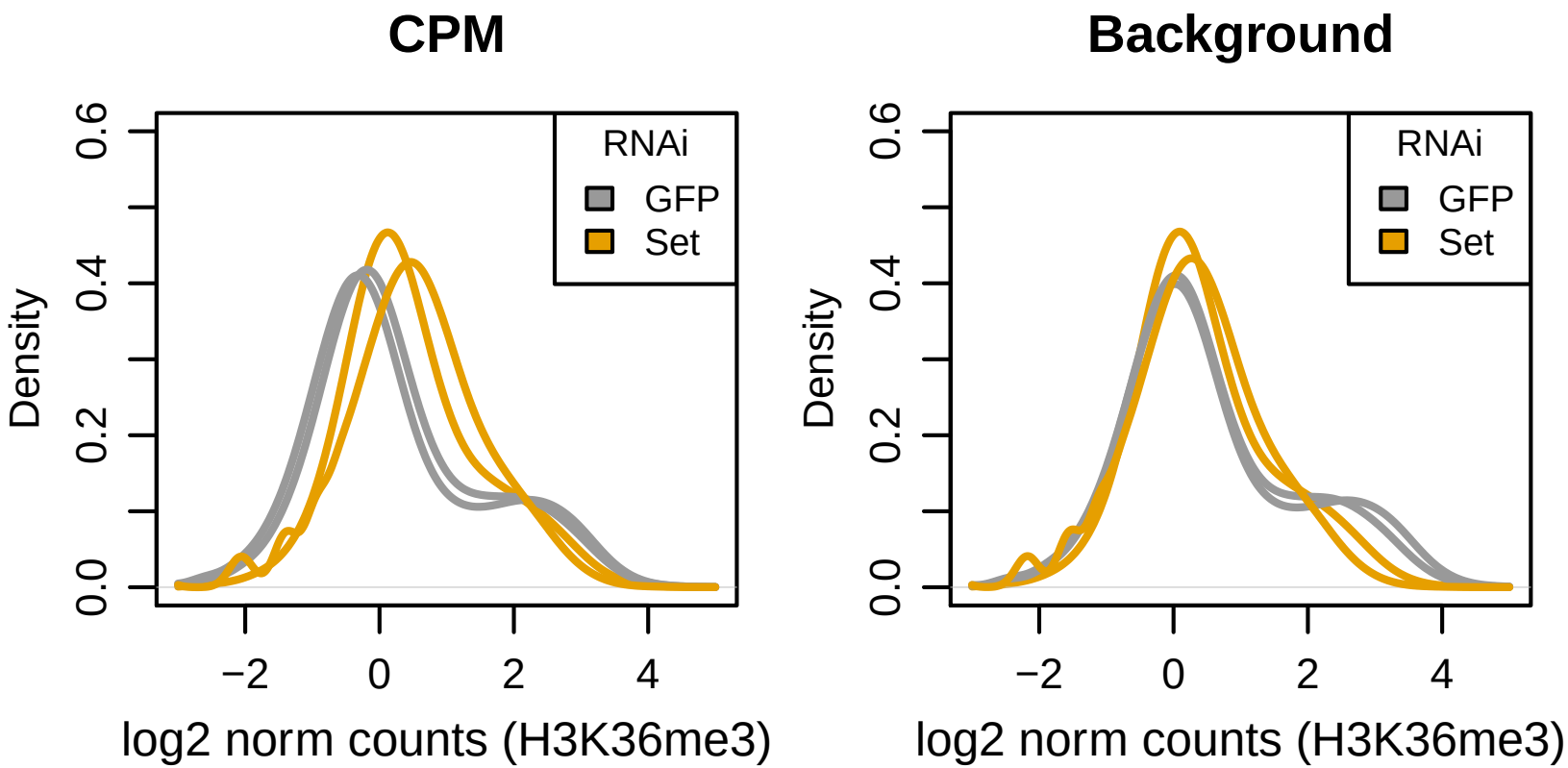
ChIP-seq Normalization

- **Sub-sampling**: random selection of certain number of reads
- **Counts Per Million (CPM)**: divide by the total number reads
- **Background**: remove compostional bias
- **Spike-In**: add constant amount of foreign chromatin

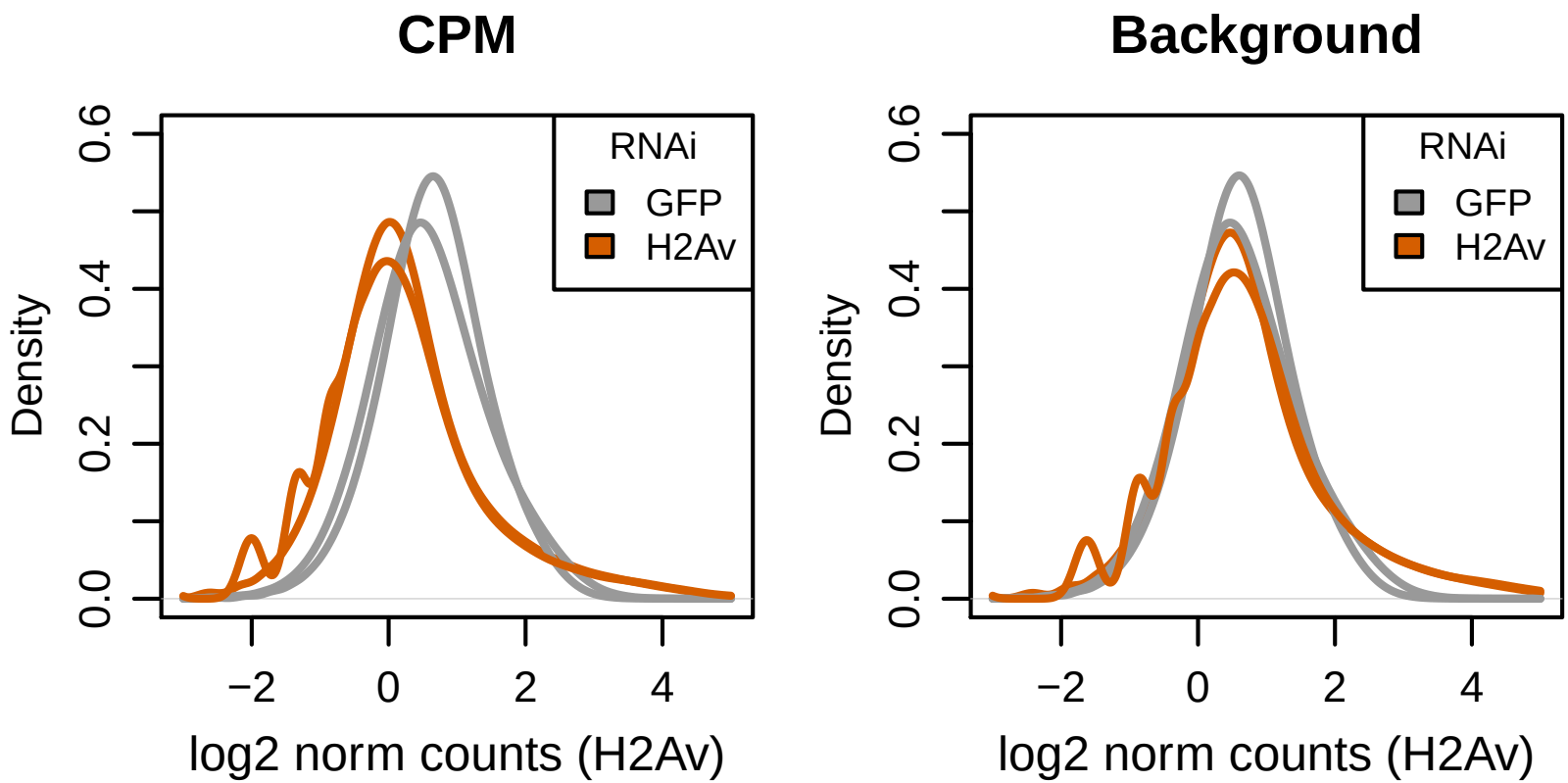
Background Normalization

- use large bins (10 kb)
- TMM - trimmed mean of M-values
- trim away extreme values
- Bioconductor: csaw package (Lun and Smyth)

Background Normalization



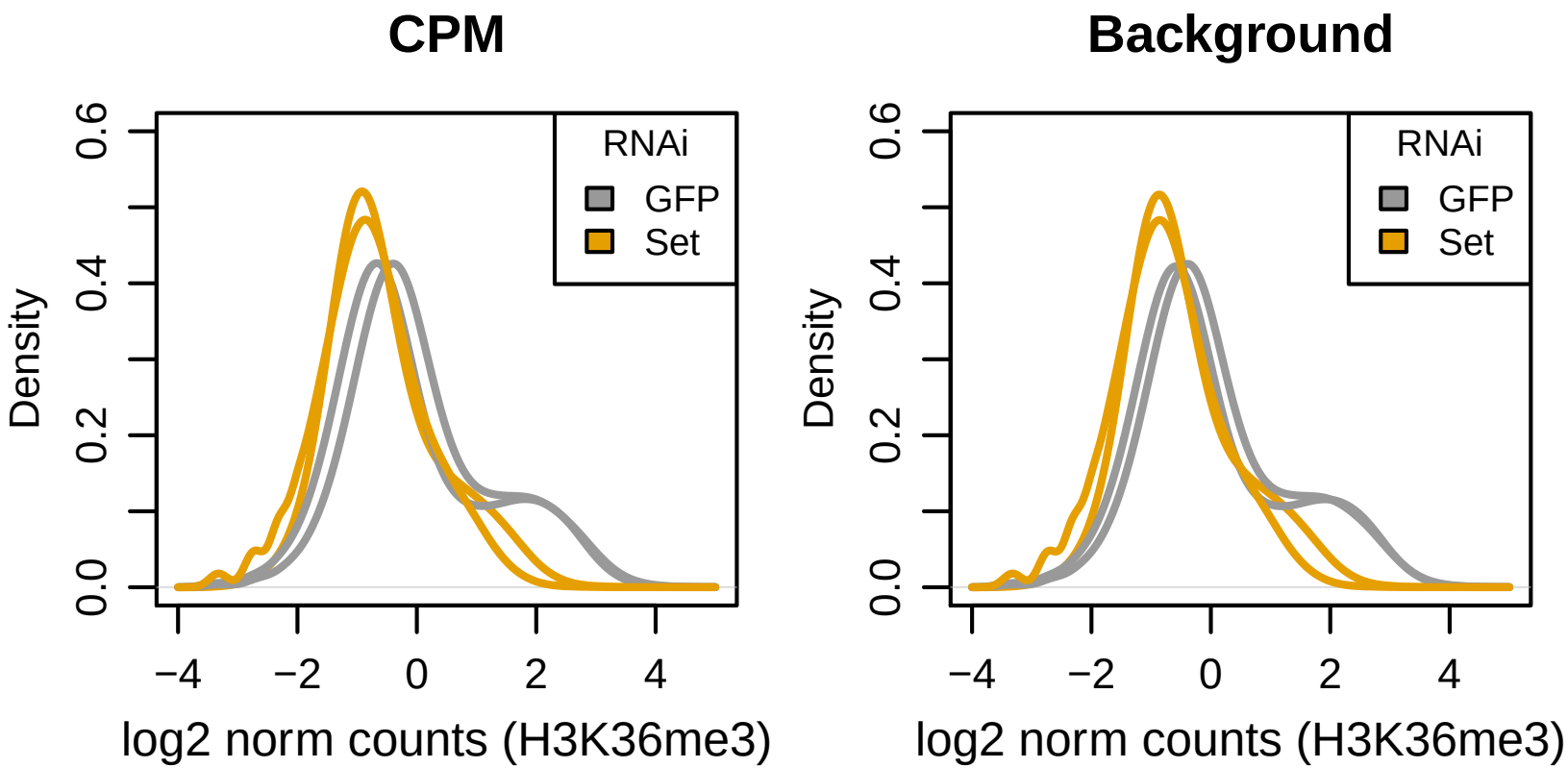
Background Normalization



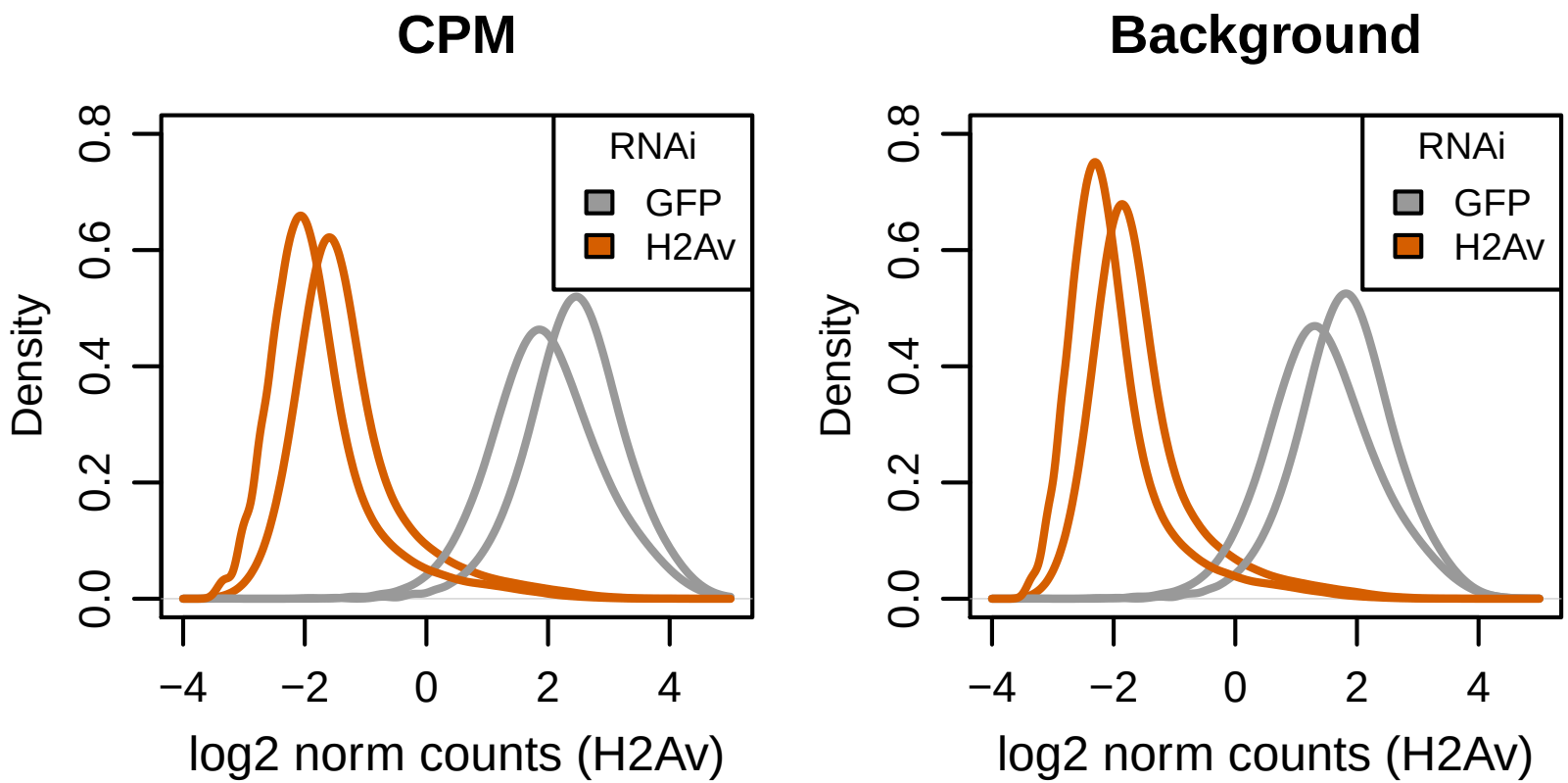
Spike-In Normalization

- Spike-In chromatin:
  - synthetic
  - different species
- Cell number and chromatin amounts have to be constant!
- Apply CPM or BG normalization on Spike-In reads

Spike-In Normalization



Spike-In Normalization



Spike-In Normalization

- When to use Spike-Ins?
  - global effect
  - effect has to be larger than variability
  - more replicates might be required

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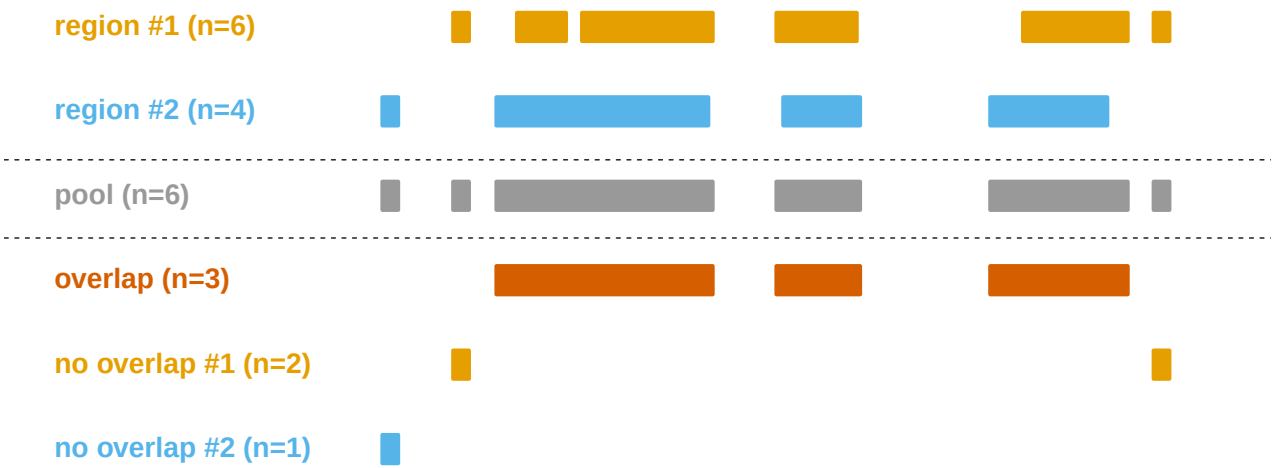
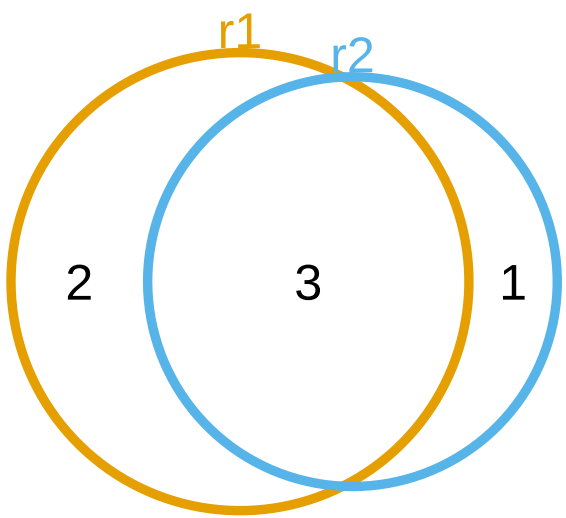
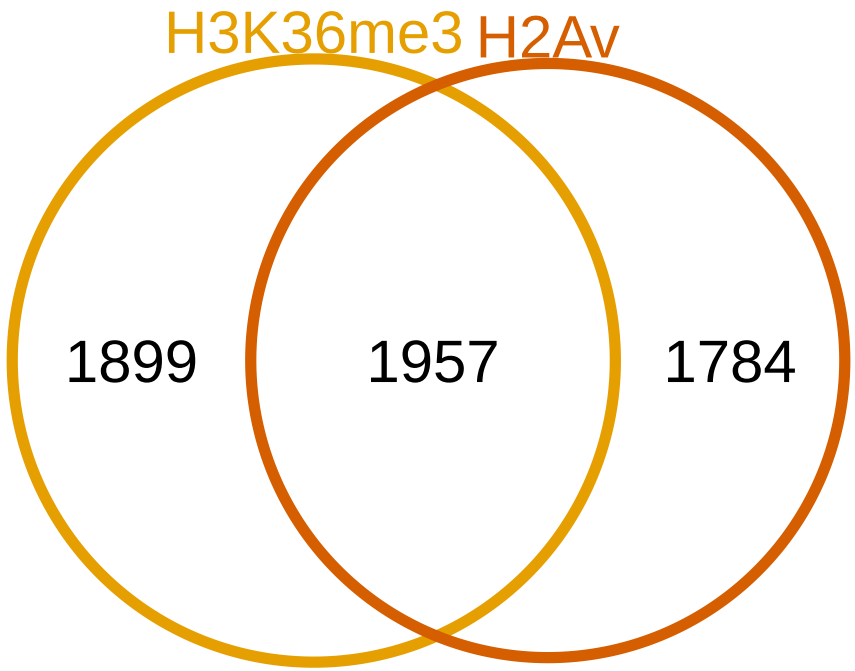
Peak Overlaps

- overlap counting rules





Peak Overlaps



- reviewers question: is this significant?

Peak Overlaps

- 2x2 contingency table

H2Av / H3K36me3	Yes	No
Yes	1957	1784
No	1899	NA

- what should be the number of unbound regions?

- use gene-based approach (unbound genes)?
- formula?

$$n = \frac{GenomeSize * (Fraction_{coding} + Fraction_{regulatory})}{(2 * PeakWidth)}$$

Peak Overlaps

H2Av / H3K36me3	Yes	No
Yes	1957	1784
No	1899	8966

Odds Ratio 95% CI = 4.77 - 5.62

Fisher´s exact test p-value $<$ 2.2e-16

- What is wrong here?

Peak Overlaps

H2Av / H3K36me3	Yes	No
Yes	1957	1784
No	1899	2000

Odds Ratio 95% CI = 1.06 - 1.27

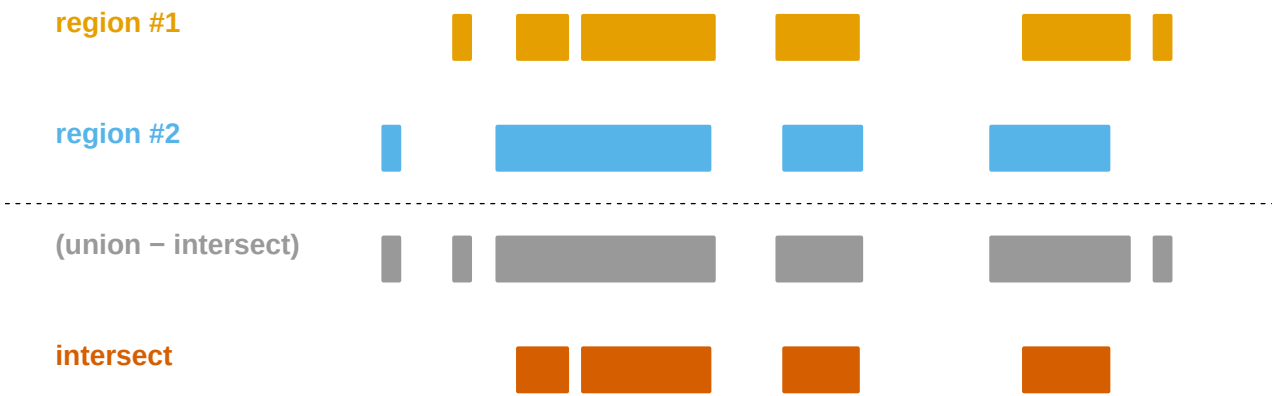
Fisher´s exact test p-value $<$ 1.7e-03

- What is wrong here?

Peak Overlaps

- Fisher´s exact test
  - hard to interpret such p-values
  - p-value is highly dependent on ‘N’
  - main problem: ‘N’ is number of peaks
  - peaks are likely not independent
  - no information about replicates
  - NOT recommended

Peak Overlaps



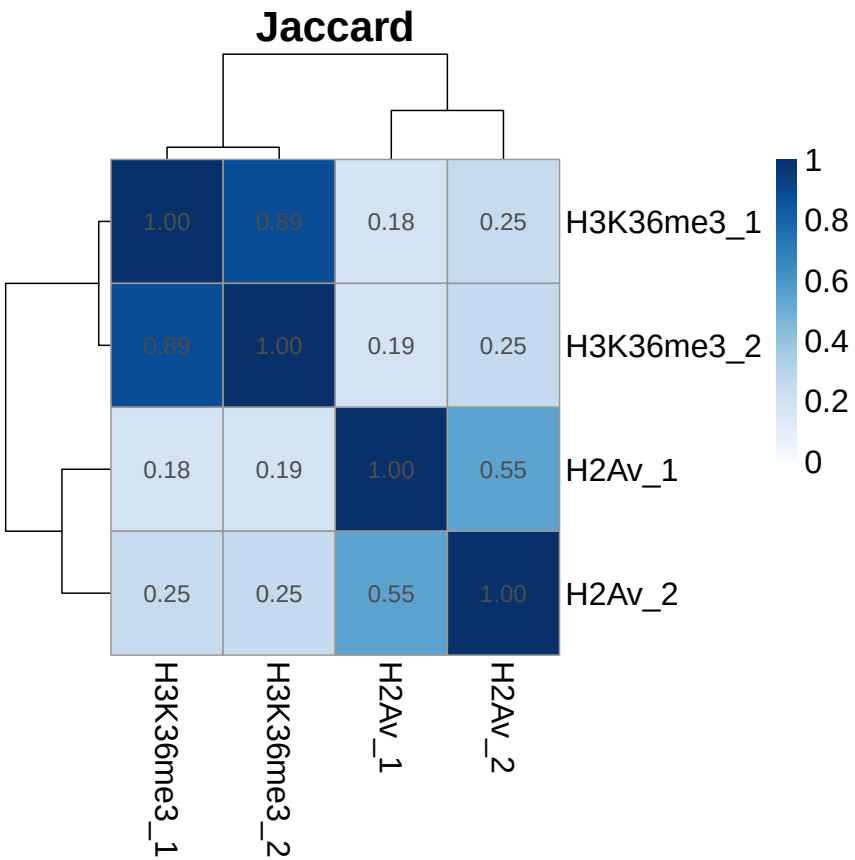
- Jaccard Similarity Index

$$Jaccard = \frac{Length_{intersect}}{Length_{union} - Length_{intersect}}$$

- Value: 0 - 1
- Example: 0.69

Peak Overlaps

- H3K36me3 vs H2Av



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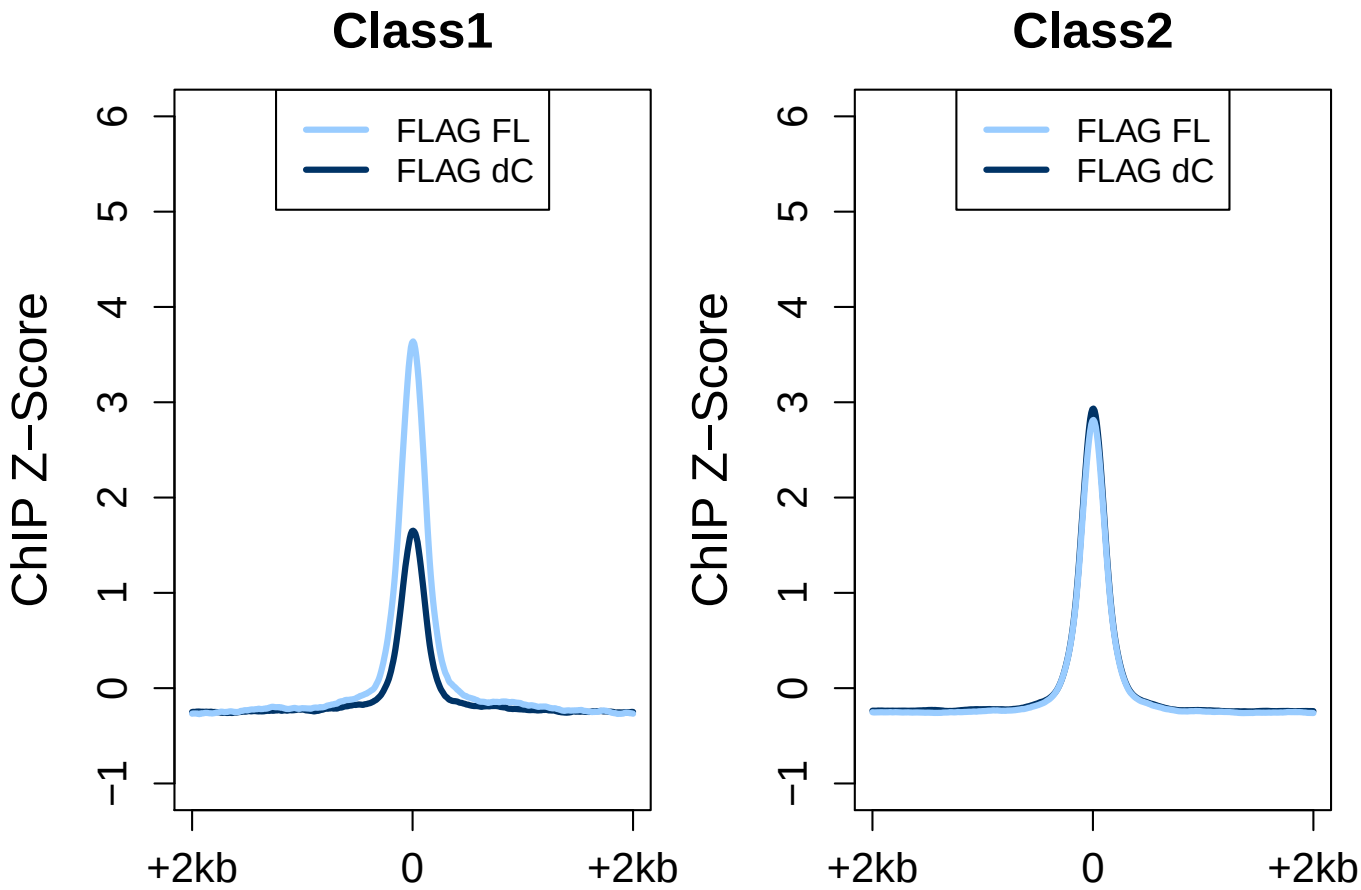
Statistical Analysis



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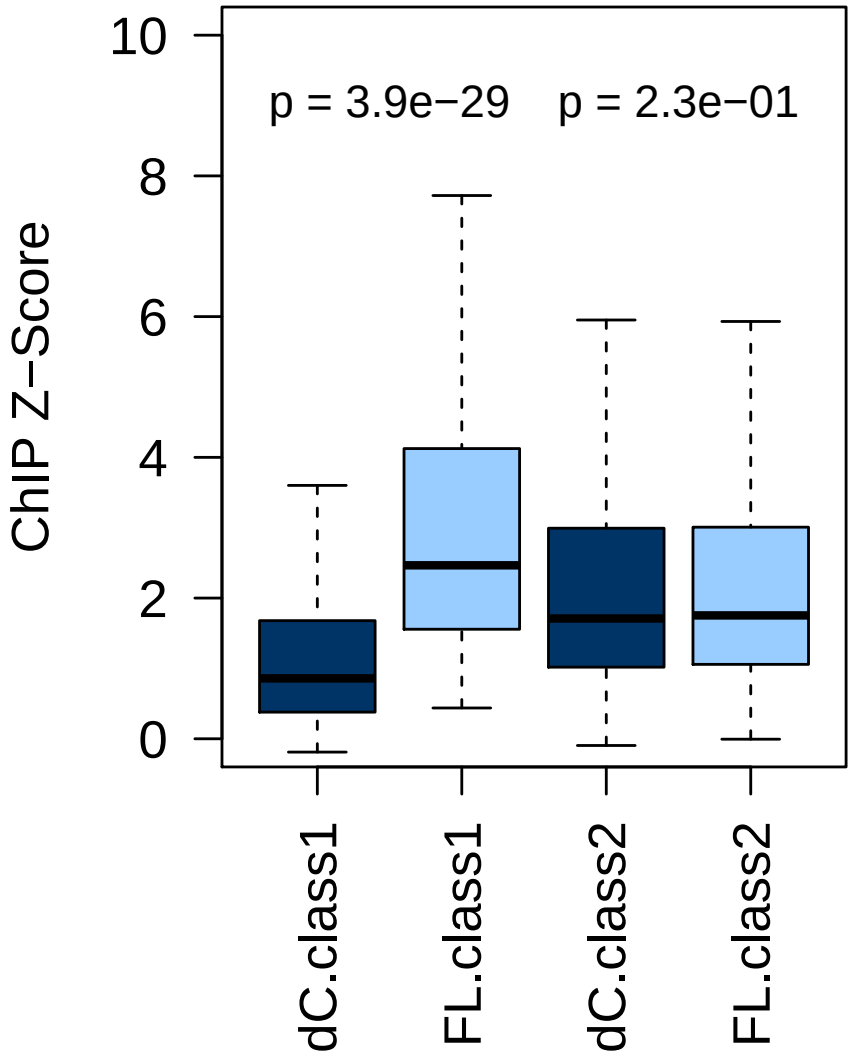
Statistical Analysis



- What is wrong here?

Statistical Analysis

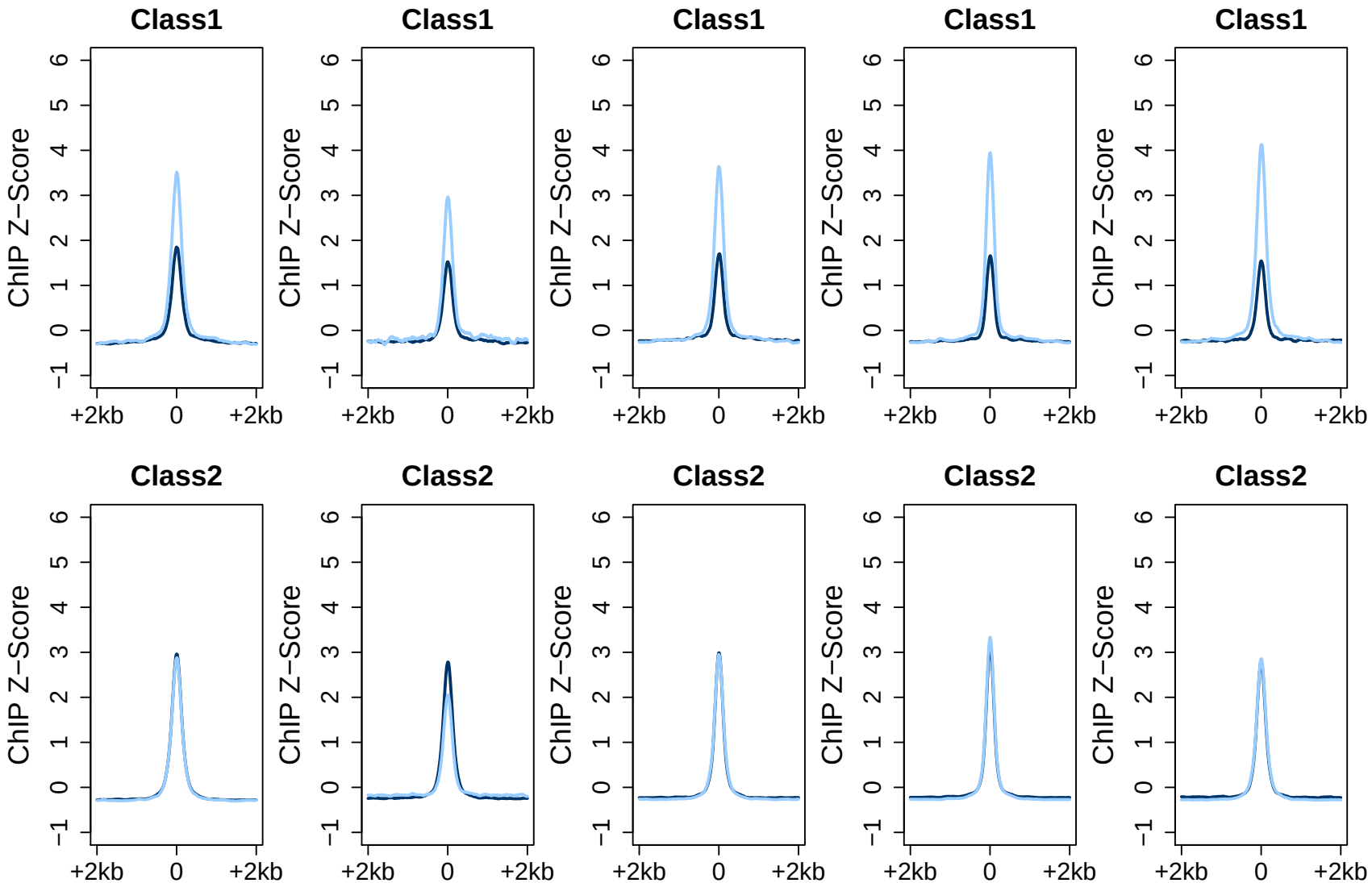
- Wilcoxon rank sum test
  - What is N?

$$N_{class1} = 230, N_{class2} = 2067$$

  - peaks are likely not independent
  - no replicate information!

  - NOT recommended

Statistical Analysis



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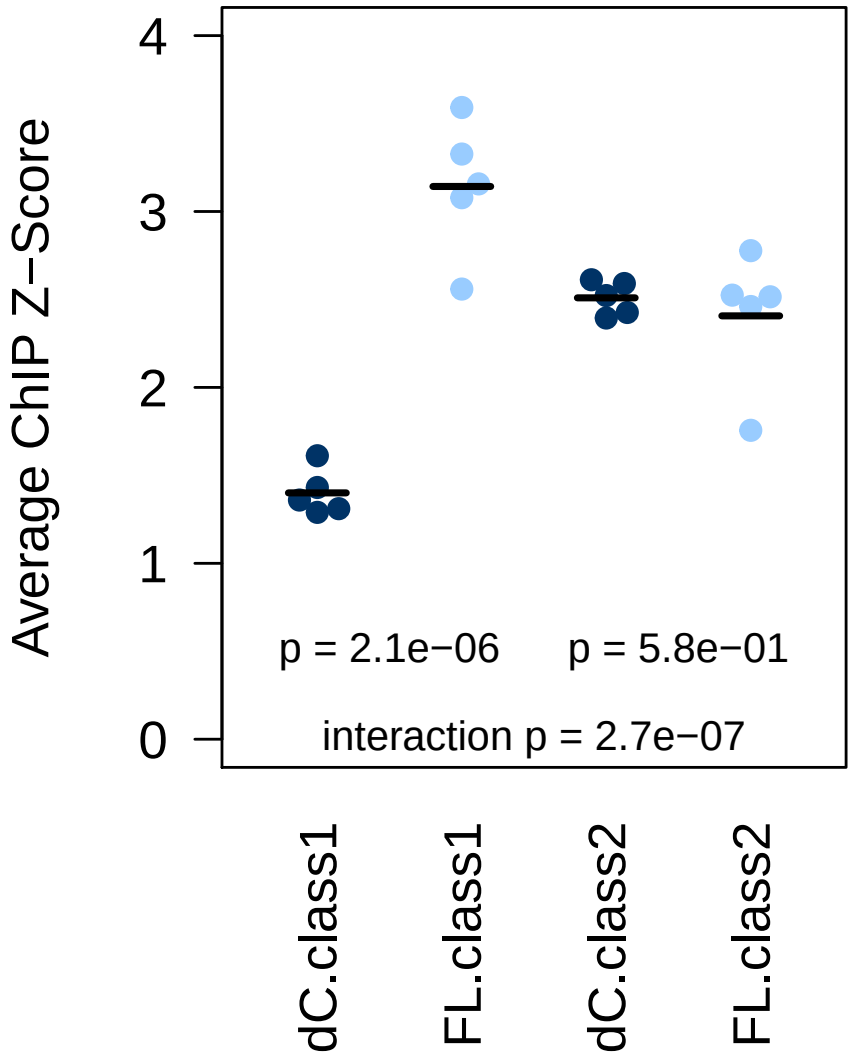

- linear mixed effect model

Acknowledgements

- **BMC, Bioinformatics**
  - Tobias Straub
- **BMC, Molecular Biology**
  - Alessandro Scacchetti
  - Andrea Lukacs
  - Catherine Regnard