Selected Topics in ChIP-seq data analysis

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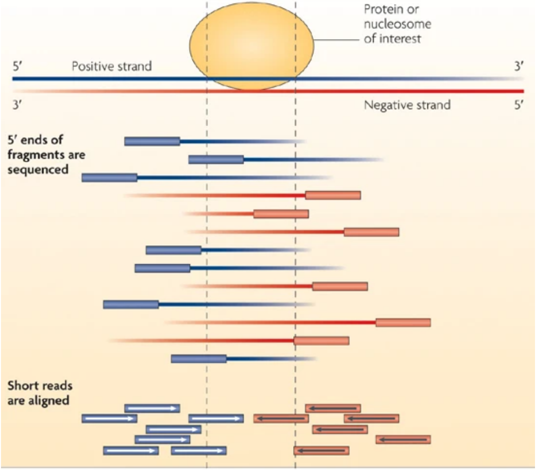
03.03.2021

Overview

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

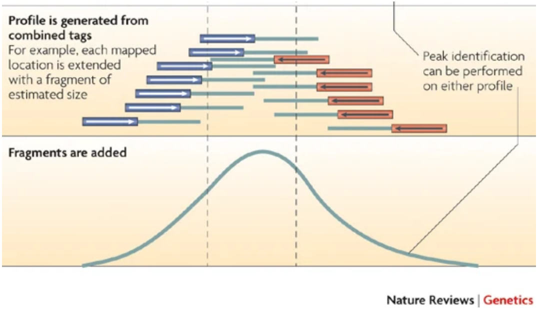
Source: https://github.com/tschauer/ChIPseq_Talk

Introduction


Protein or nucleosome of interest
5′
Positive strand
3′
3′
Negative strand
5′
5′ ends of fragments are sequenced
Short reads are aligned

Peter J. Park, 2009

Introduction


Profile is generated from combined tags
For example, each mapped location is extended with a fragment of estimated size
Peak identification can be performed on either profile
Fragments are added
Nature Reviews | Genetics

Peter J. Park, 2009

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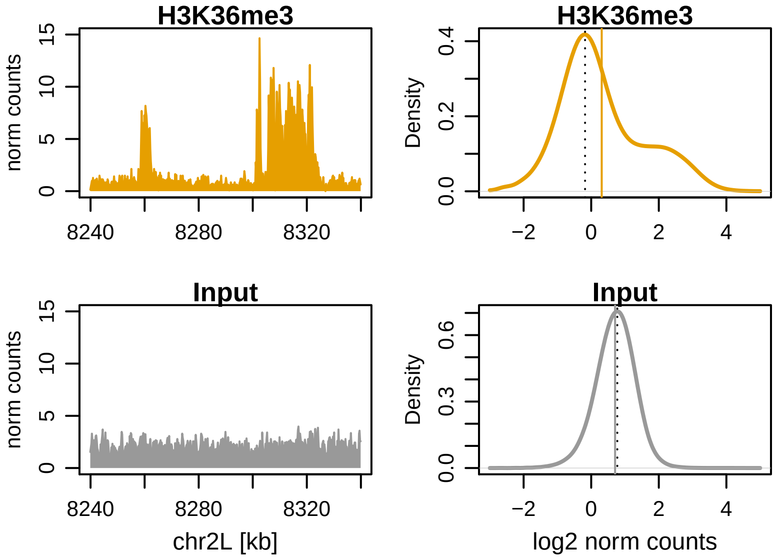
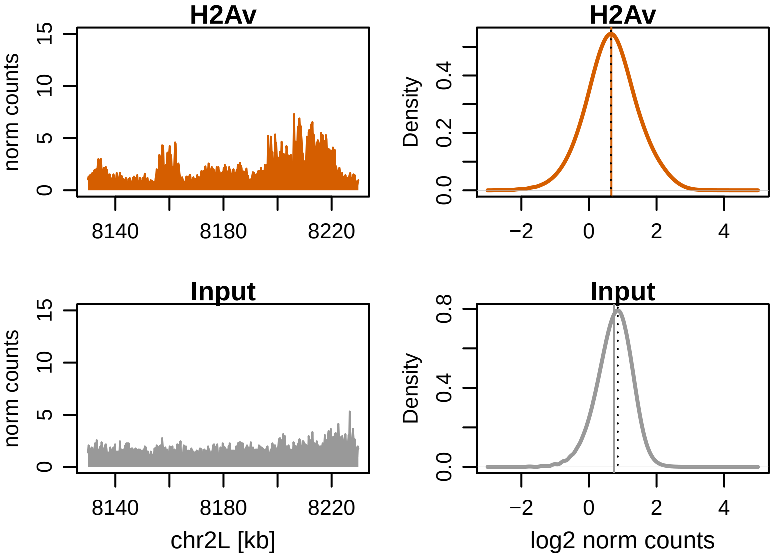
ChIP Coverage



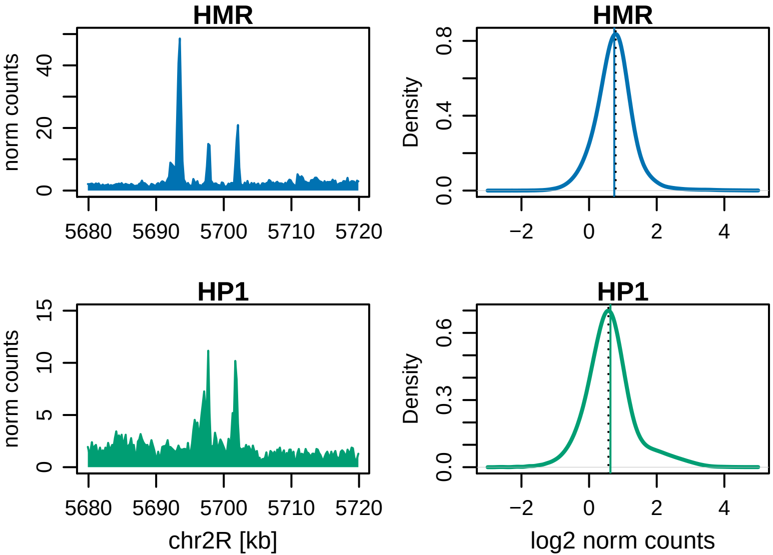
Catherine Regnard

ChIP Coverage



Alessandro Scacchetti

ChIP Coverage



Andrea Lukacs

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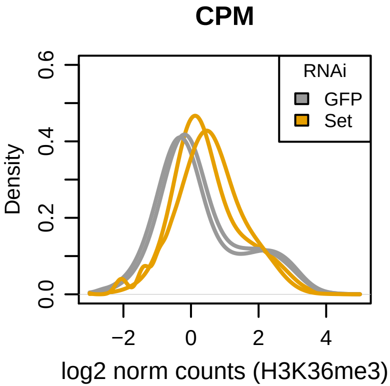
ChIP-seq Normalization

- **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

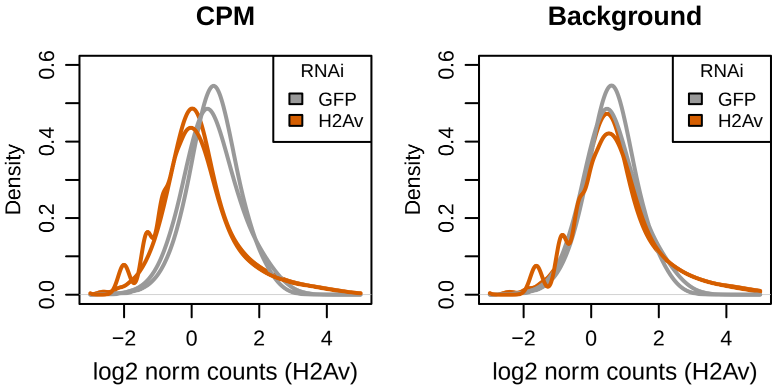
Background Normalization

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

Background Normalization



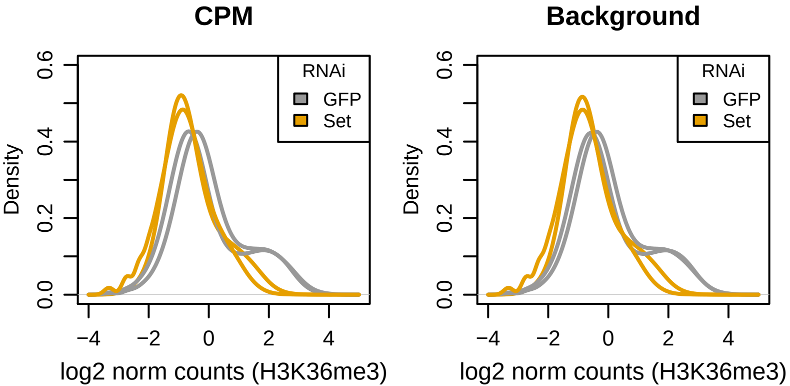
Background Normalization



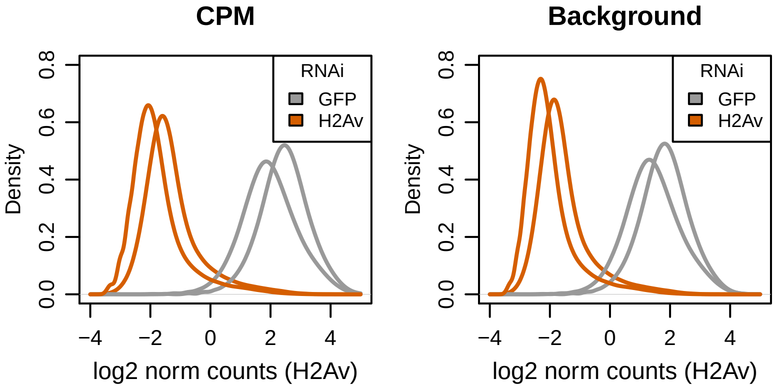
Spike-In Normalization

- 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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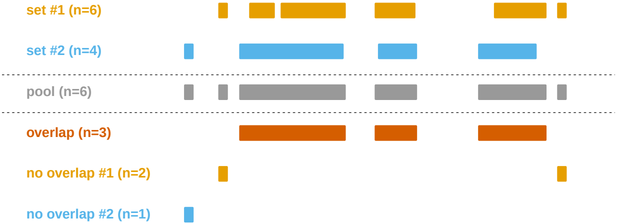
Peak Overlaps

- overlap counting rules



set #1 (n=6)

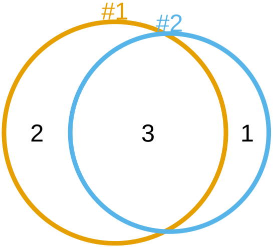
set #2 (n=4)

pool (n=6)

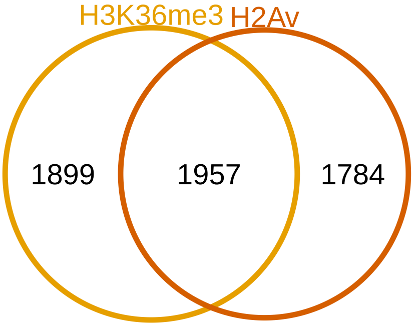
overlap (n=3)

no overlap #1 (n=2)

no overlap #2 (n=1)



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 $< 2.2e\text{-}16$

- What is wrong here?

Peak Overlaps

H2Av / H3K36me3	Yes	No
Yes	1957	1784
No	1899	2000

Odds Ratio 95% CI = 1.06 - 1.27

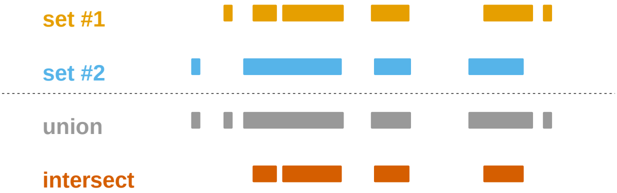
Fisher´s exact test p-value $< 1.7e\text{-}03$

- 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



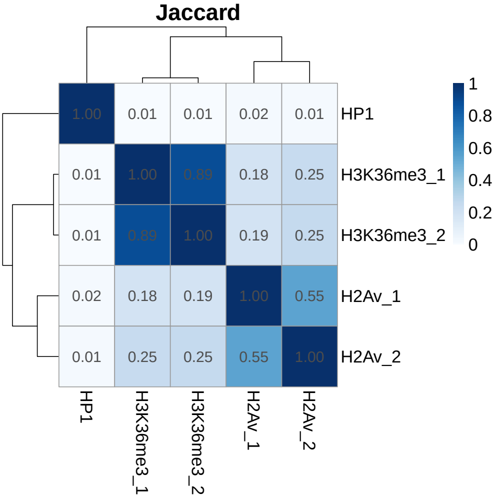
- Jaccard Similarity Index

$$Jaccard = \frac{Length_{intersect}}{Length_{set1} + Length_{set2} - Length_{intersect}} = \frac{Length_{intersect}}{Length_{union}}$$

- Value: 0 - 1
- Example: 0.69

Peak Overlaps

- H3K36me3 vs H2Av vs HP1



	HP1	H3K36me3_1	H3K36me3_2	H2Av_1	H2Av_2
HP1	1.00	0.01	0.01	0.02	0.01
H3K36me3_1	0.01	1.00	0.89	0.18	0.25
H3K36me3_2	0.01	0.89	1.00	0.19	0.25
H2Av_1	0.02	0.18	0.19	1.00	0.55
H2Av_2	0.01	0.25	0.25	0.55	1.00

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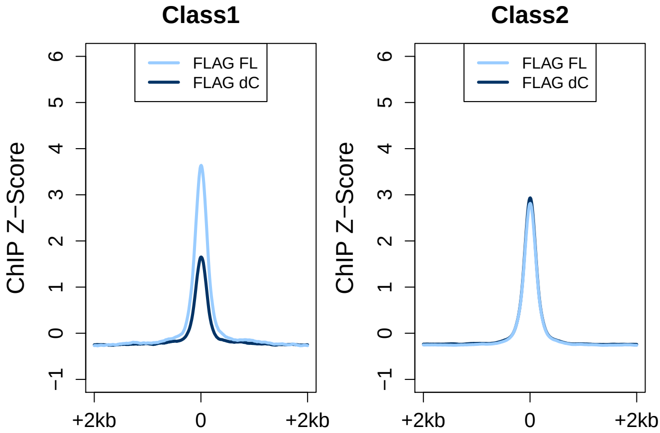
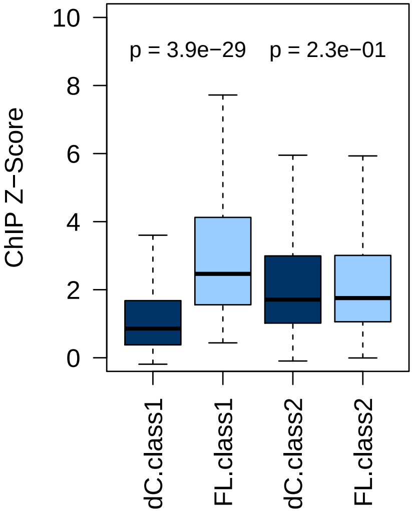
Statistical Analysis



- reviewers question: is this significant?

Statistical Analysis



- What is wrong here?

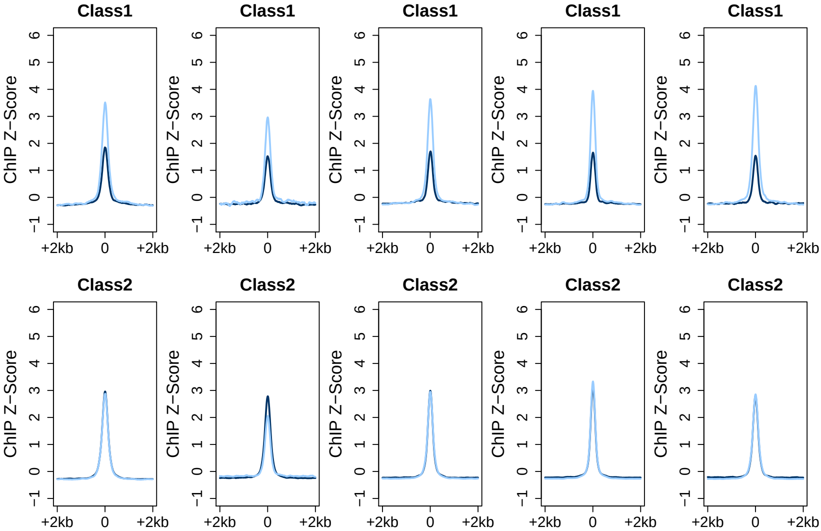
Statistical Analysis

- Wilcoxon rank sum test
  - What is N?

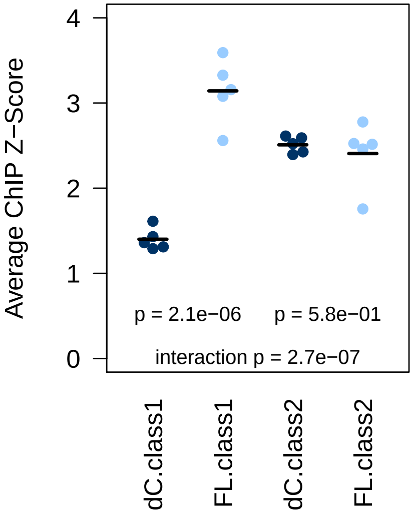
    $$N_{class1} = 230, N_{class2} = 2067$$

  - peaks are likely not independent
  - no replicate information!
  - NOT recommended

Statistical Analysis



Statistical Analysis



- linear mixed effect model

Acknowledgements

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  - Tobias Straub
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  - Alessandro Scacchetti
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SFB1064
Chromatin
Dynamics