Exploring and Understanding ChIP-Seq data

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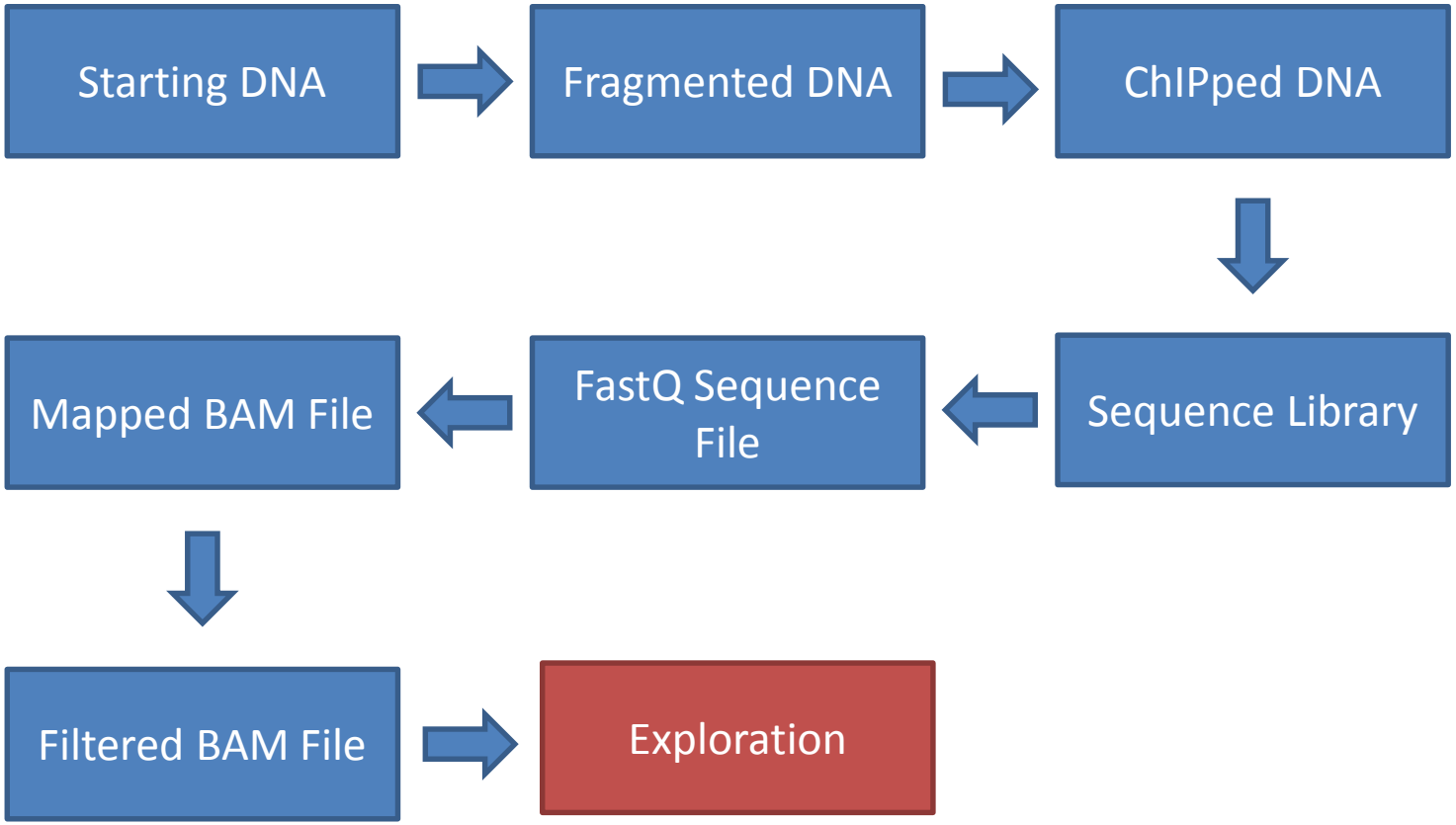
Data Creation and Processing



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Some Basic Questions

- Is there any enrichment?
- What is the size / patterning of enrichment?
- How well are my controls behaving?
- What is the best way to quantitate this data?
- Are there any technical artefacts?

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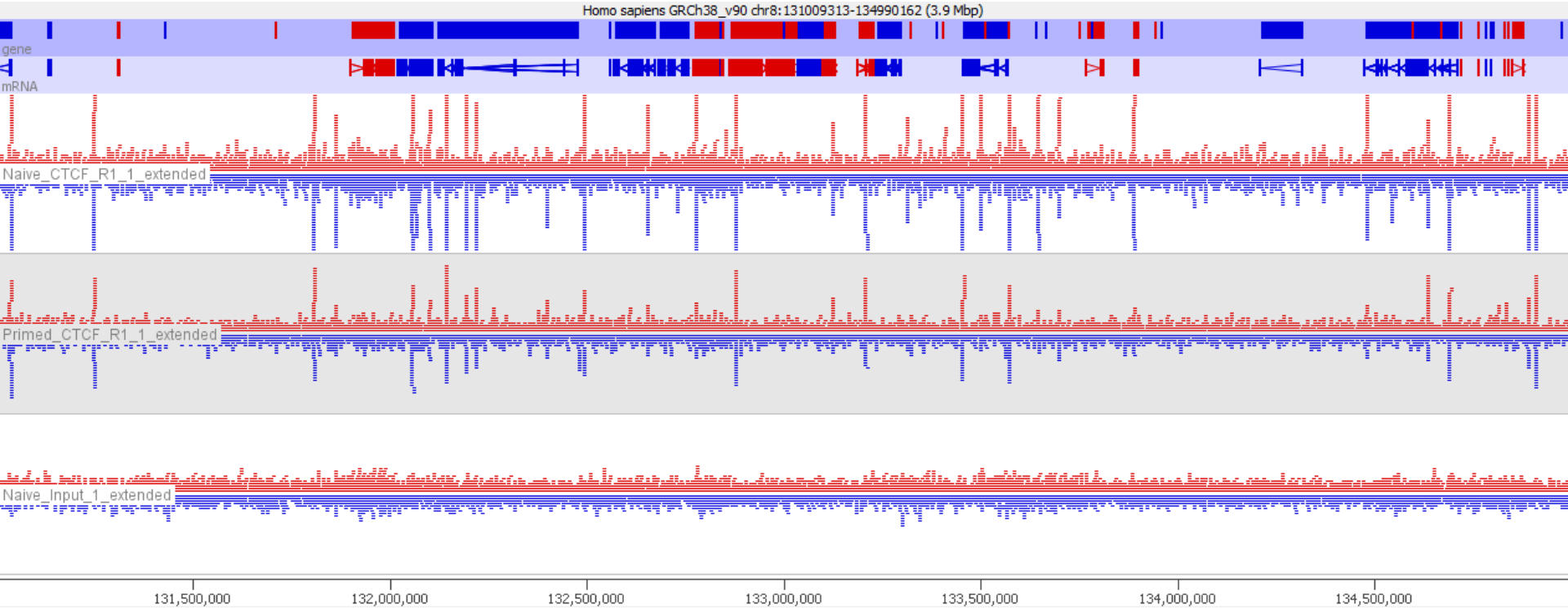
Start with a visual inspection



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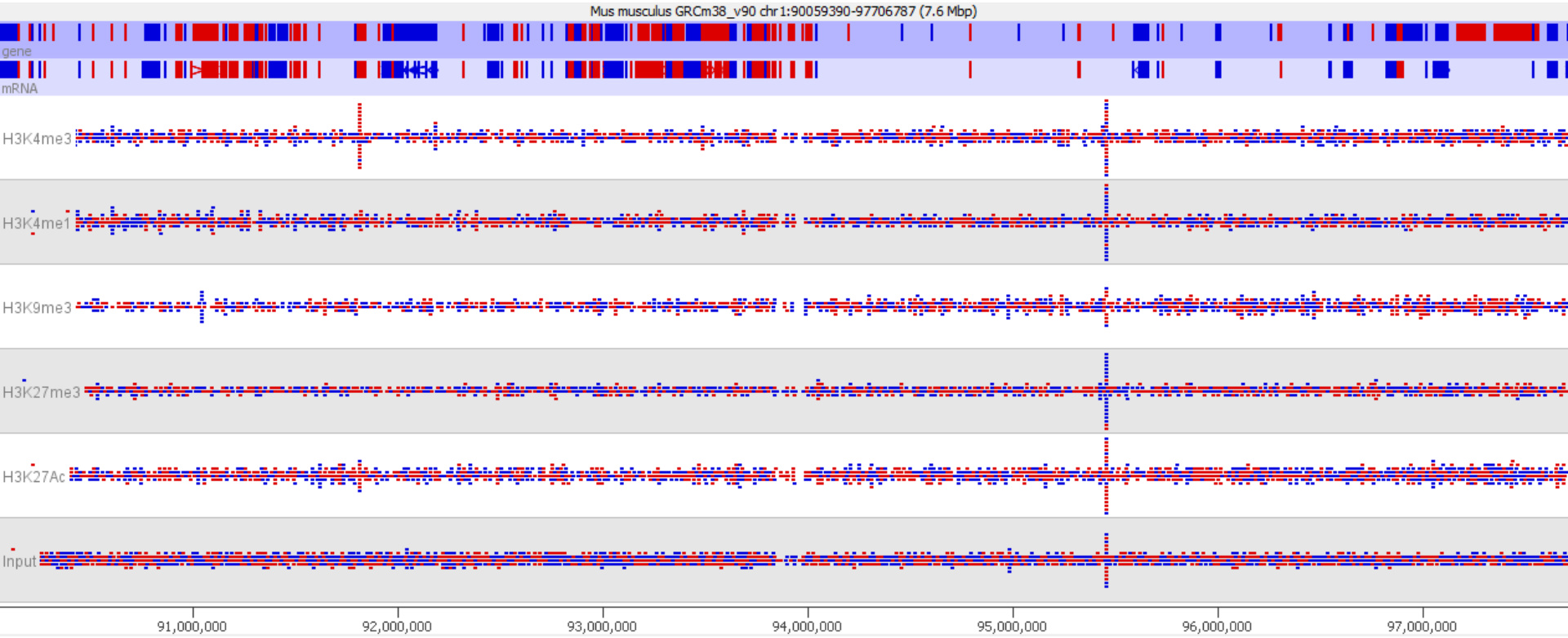

- Is there any enrichment?
- What is the size / patterning of enrichment?
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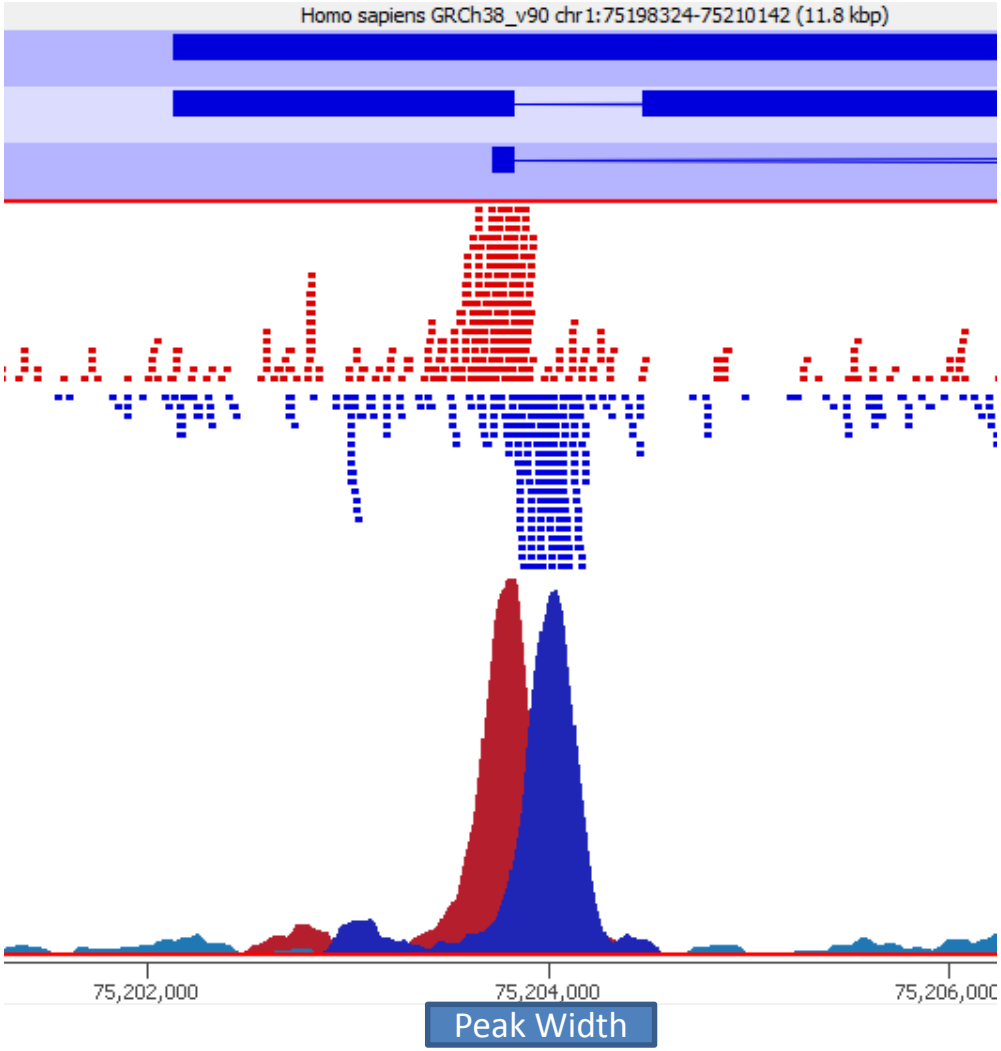
Extending reads if necessary



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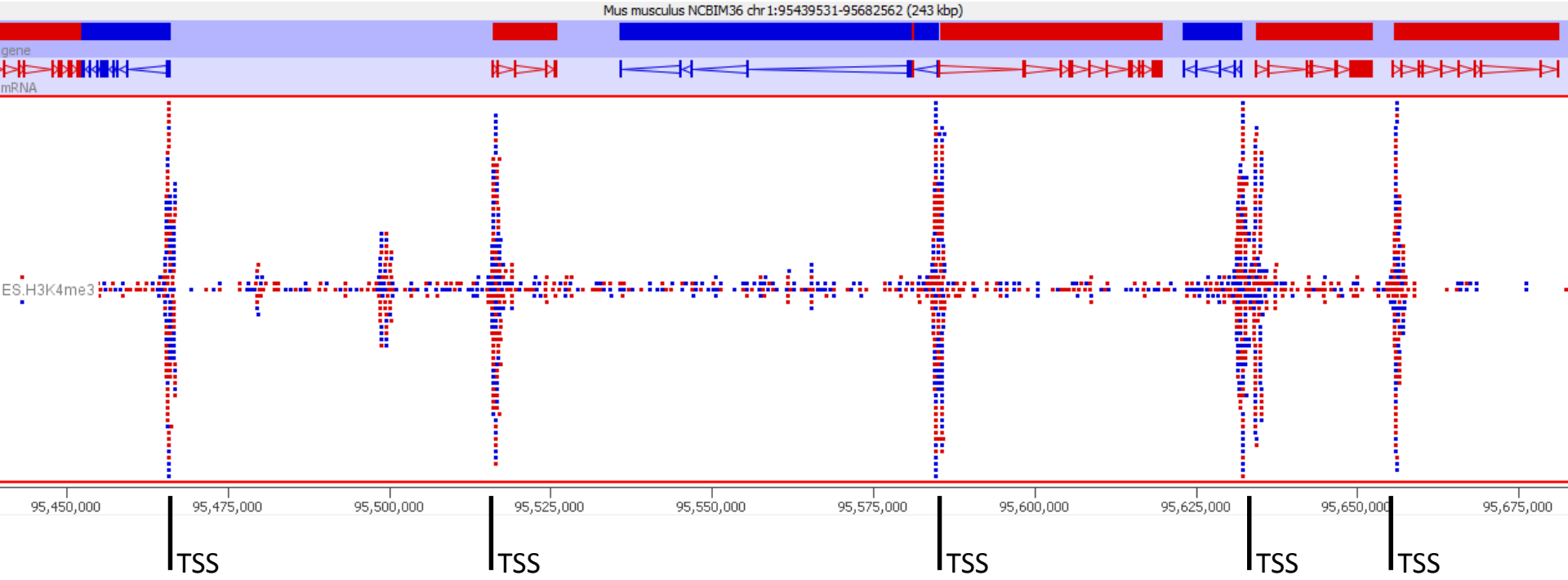
For point enrichment, insert size is roughly peak width/2

Look for peaks
Associate with features



- Are my peaks narrow or broad
- Do peak positions obviously correspond to existing features?

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Examine Controls

- IgG or other Mock IP
  - Good result is no material at all
  - Not worth sequencing. Reads are only informative if the ChIP hasn't worked.

- Input material (sonicated / Mnase etc)
  - Genomic library - everywhere equally
  - Technical issues can cause variation

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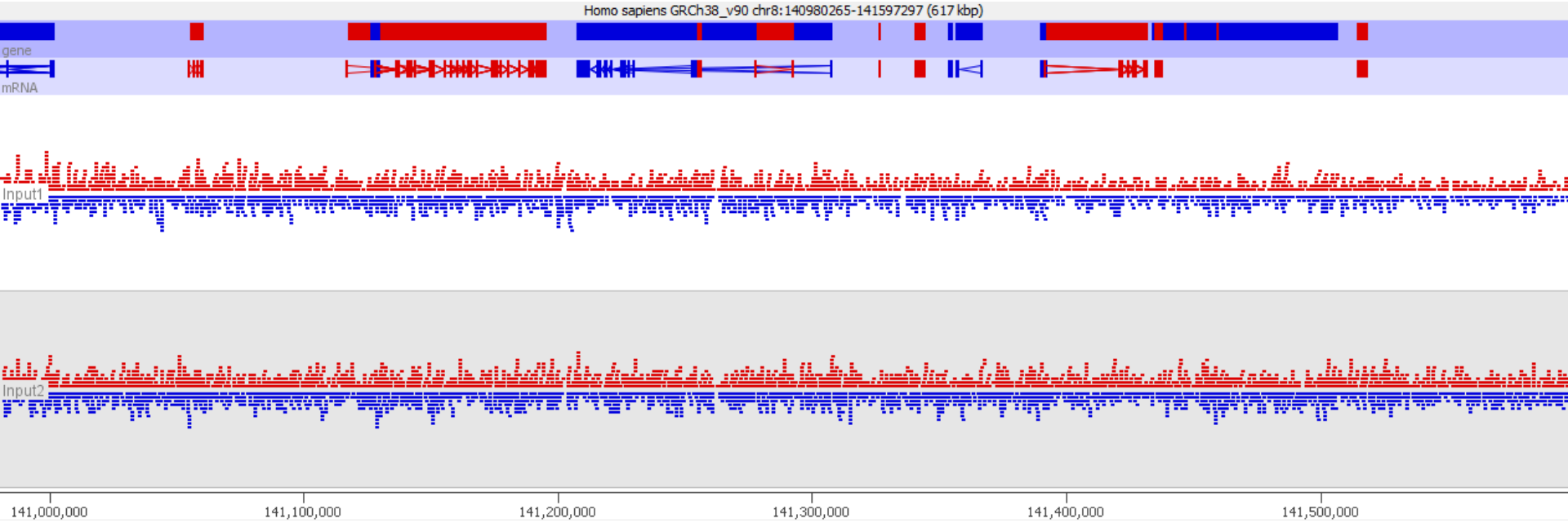
Examine Controls



- Does the coverage look even
- If there are multiple inputs to do they look similar

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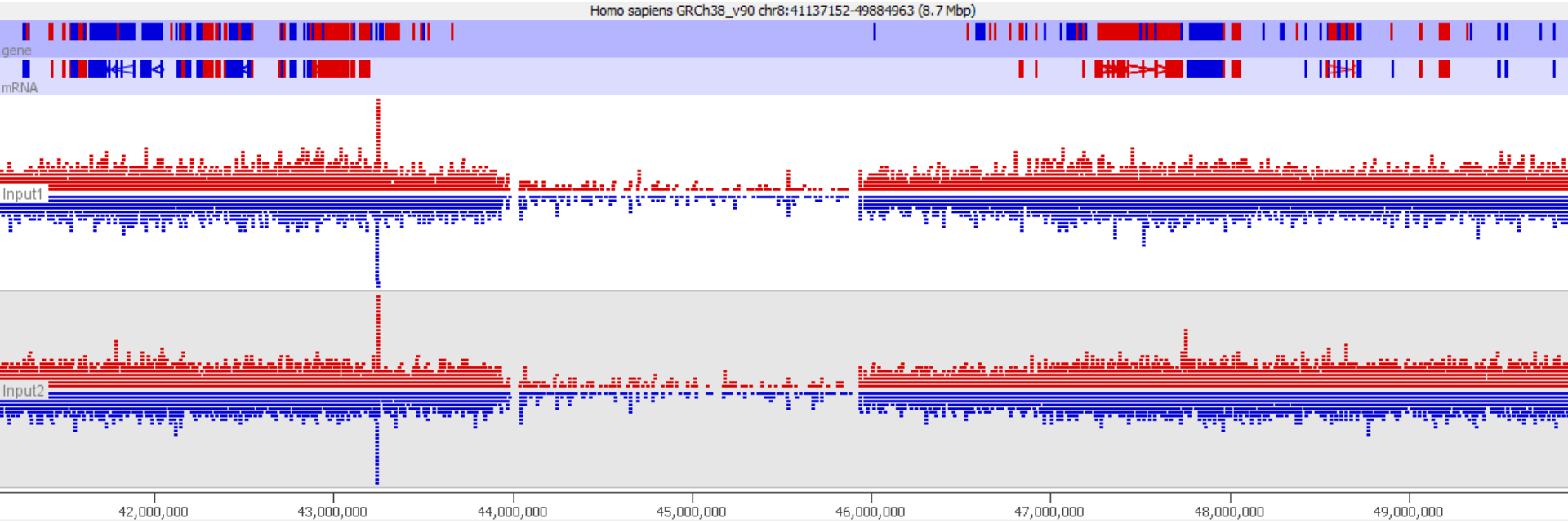
Examine Controls



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Why do controls misbehave?

- Low coverage
  - Repetitive unmappable regions
  - Holes in the assembly
- High coverage
  - Mismapped reads from outside the assembly
- Biases
  - DNA stability
  - GC content
  - Segmental Duplication

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Types of input problem

- Categorical
  - Mismapped reads
  - Indicates that the region can't be trusted
  - Blacklist and remove - don't try to correct

- Quantitative
  - Other biases (most often GC)
  - Some potential to correct, but difficult
  - Hopefully consistent, so will cancel out

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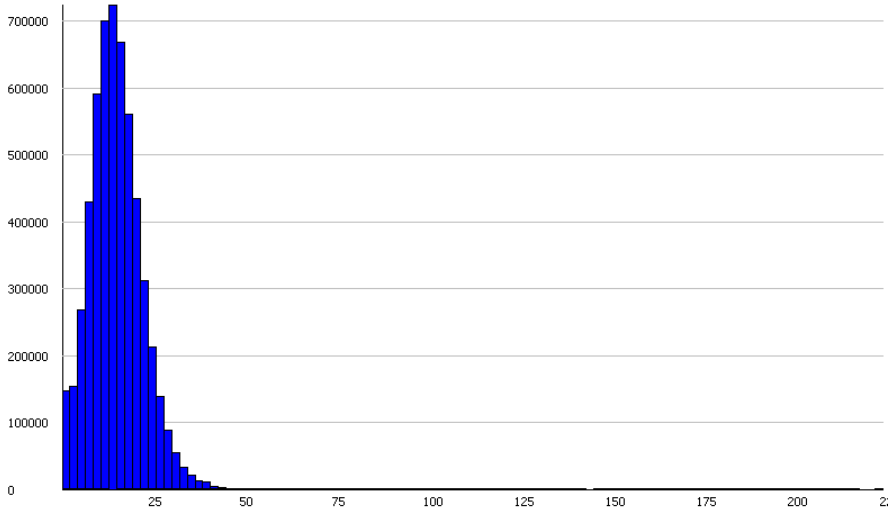
Making Blacklists

- Unusual Coverage
  - Outlier detection (boxplots etc.)
  - Often only filter over-representation (maybe also zero counts)

- Pre-built lists
  - Large projects often build these (ENCODE)
  - Not for all species



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Pre-Existing Blacklists

- ENCODE
- modENCODE
- UCSC

frontiers in GENETICS

TECHNOLOGY REPORT ARTICLE
published: 10 April 2014
doi: 10.3389/fgene.2014.00075

Impact of artifact removal on ChIP quality metrics in ChIP-seq and ChIP-exo data

Thomas S. Carroll[1*†], Ziwei Liang[2†], Rafik Salama[1†], Rory Stark[1] and Ines de Santiago[1*]

[1] Cambridge Institute CRUK, University of Cambridge, Cambridge, UK
[2] Lymphocyte Development, MRC Clinical Sciences Centre, Imperial College, London, UK

- Check assembly versions

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https://sites.google.com/site/anshulkundaje/projects/blacklists

Comparison of samples

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Initial Quantitation

- Always start with a simple unbiased quantitation (not focussed on features/peaks)
- Tiled measures over the whole genome
  - Use approximate insert size as window size
  - Something around 500bp is normally sensible
- Linear read count quantitation corrected for total library size

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Quantitation Questions

- Initially similar to what we did with the raw data, but with some values behind it
  - Can we observe enrichment in our ChIP above our controls
  - Do we see similar enrichment between replicates, or between conditions

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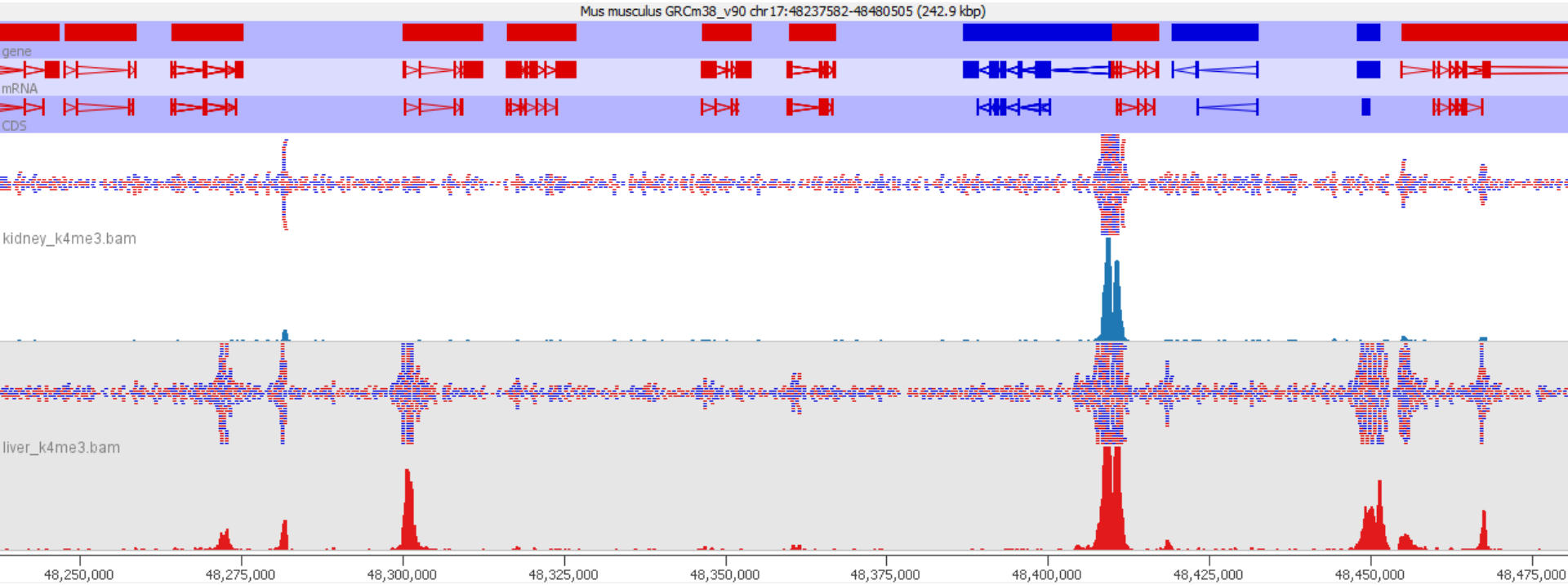
Compare samples

Visual comparison against raw data



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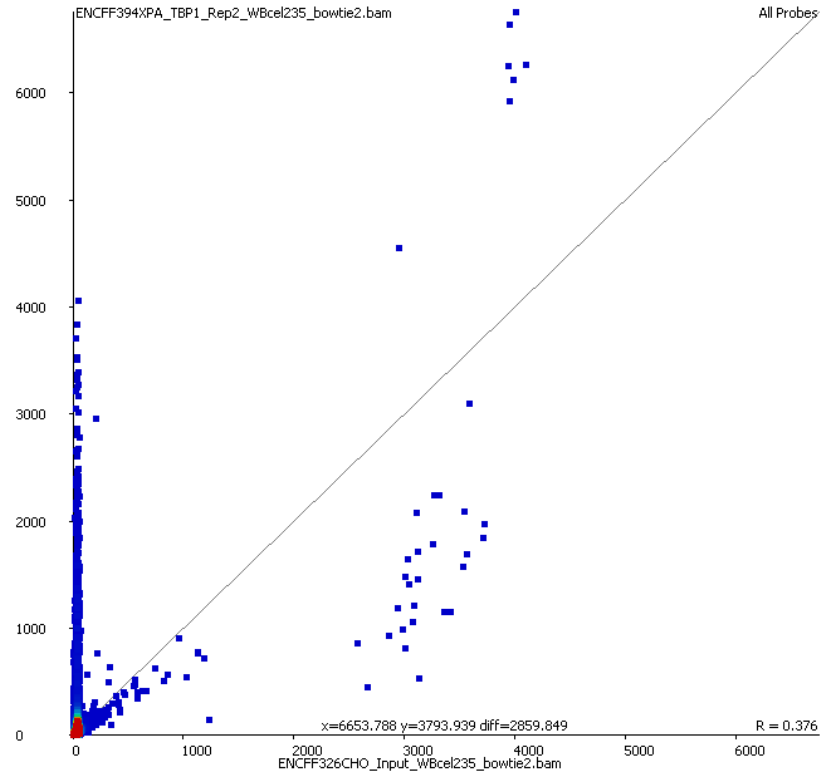
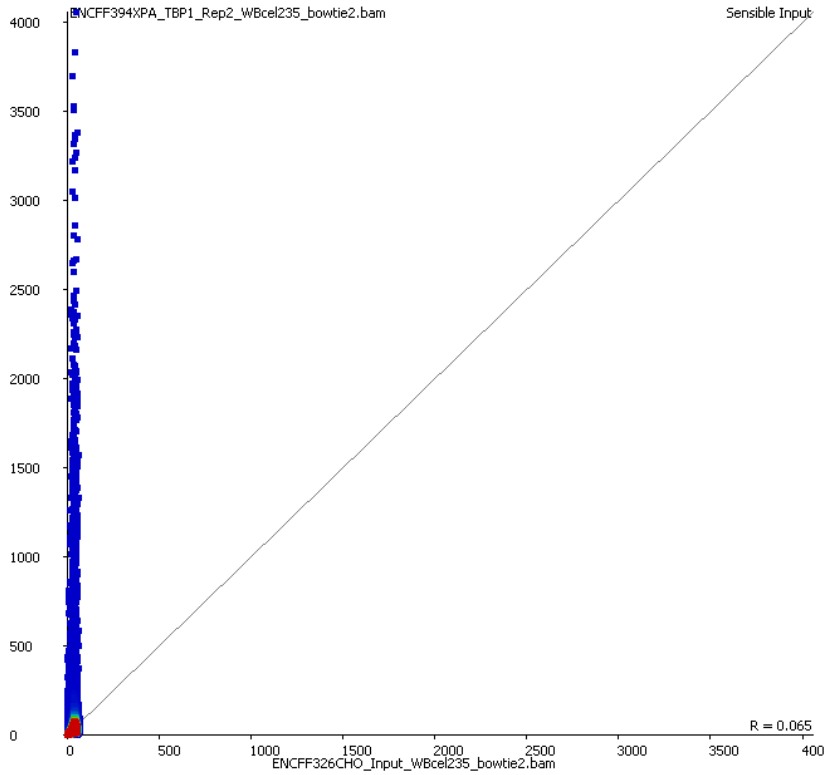
- Similar apparent overall enrichment
- Any obvious differences

Compare samples

Scatterplot input vs ChIP



Raw



Filtered

- Any relationship between input and ChIP
- What proportion enriched
- How enriched over input

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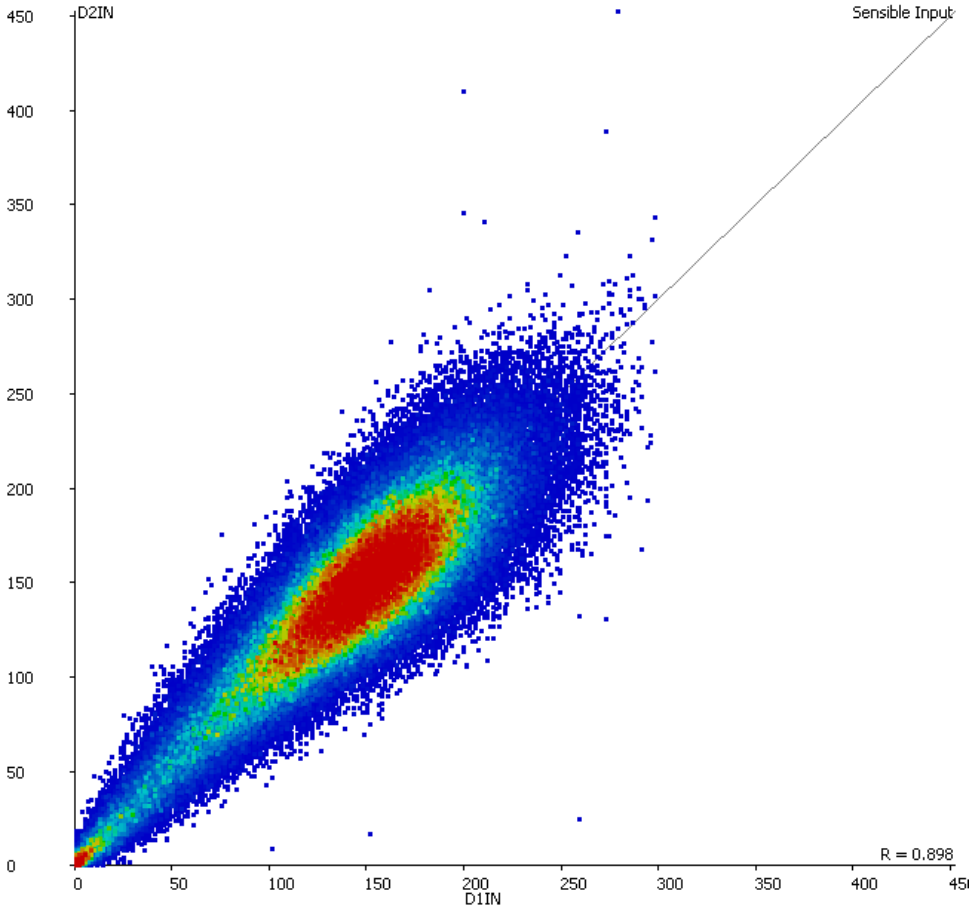
Compare samples

Scatterplot input vs input



- Any suggestion of differential biases in inputs
- Can we merge them to use as a common input

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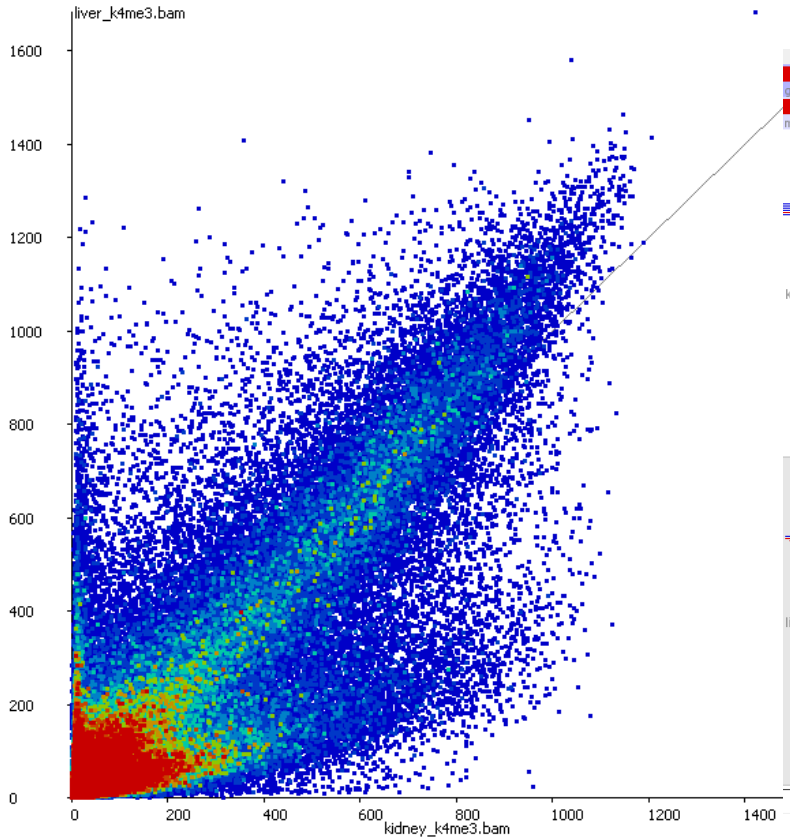
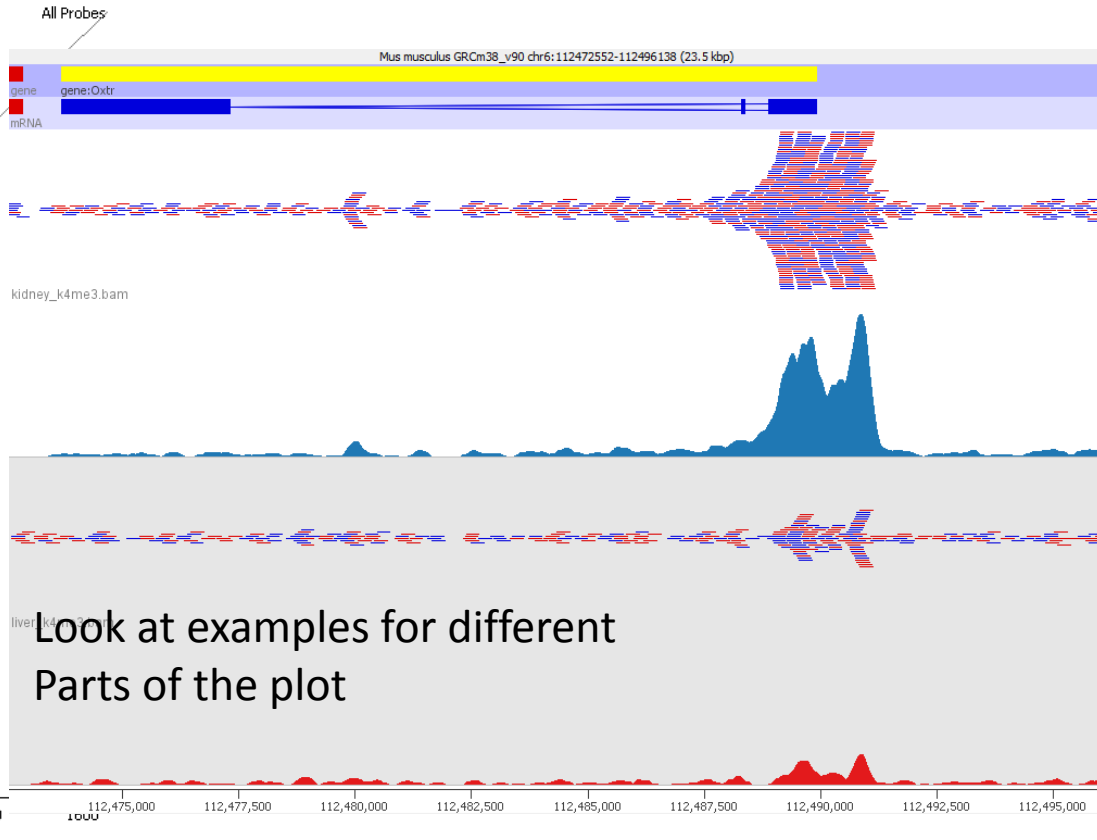
Compare samples

Scatterplot ChIP vs ChIP



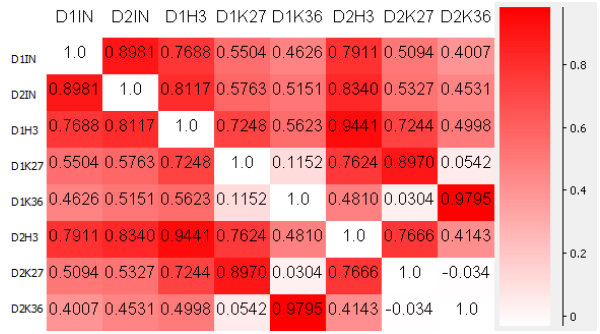


Look at examples for different Parts of the plot

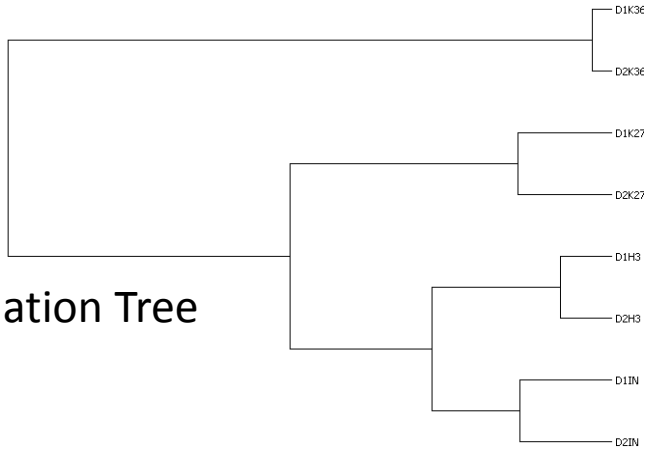
- Look for outgroups (differentially enriched)
- Compare level of enrichment (compare to diagonal)

Compare samples

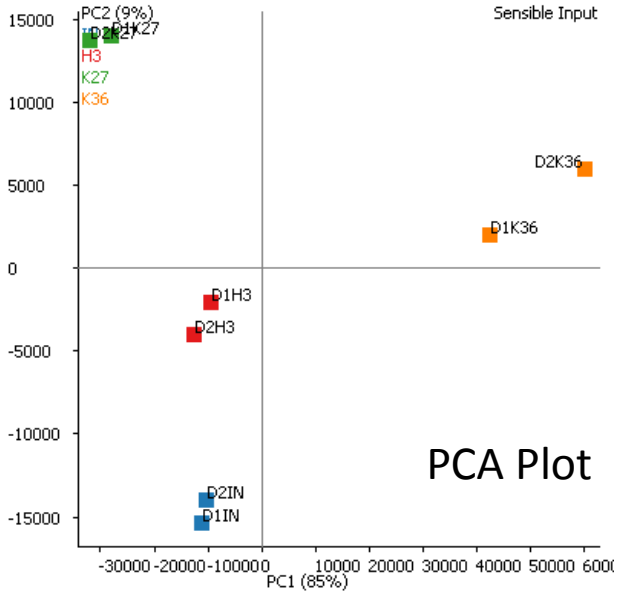
Higher level clustering



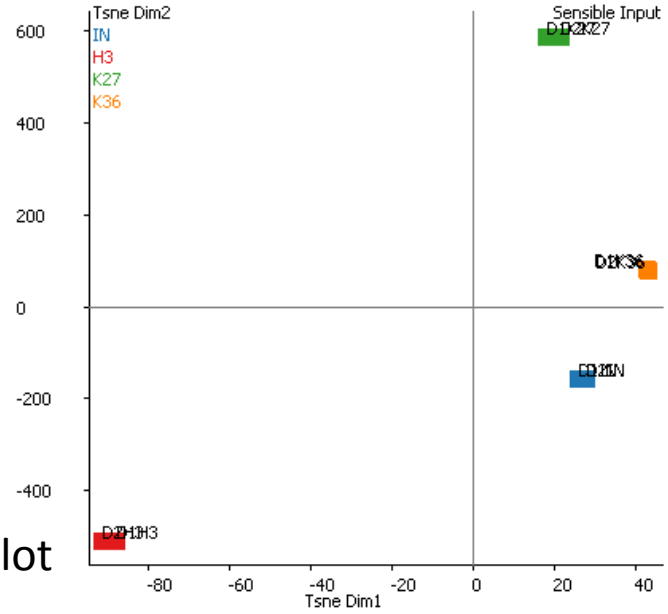
Correlation Matrix



Correlation Tree



PCA Plot



tSNE Plot

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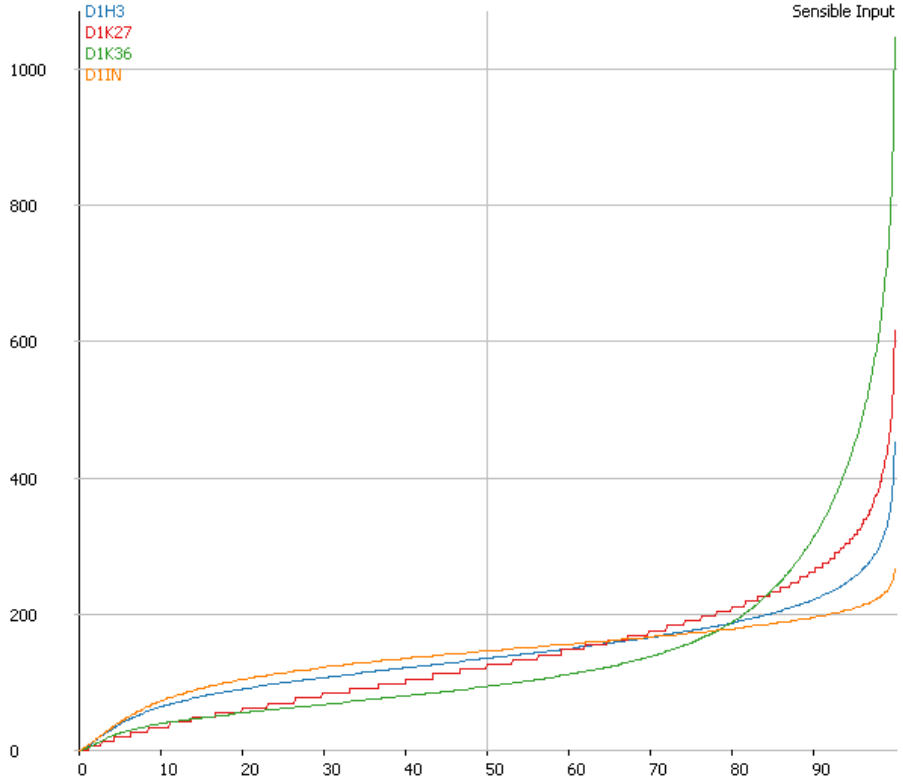
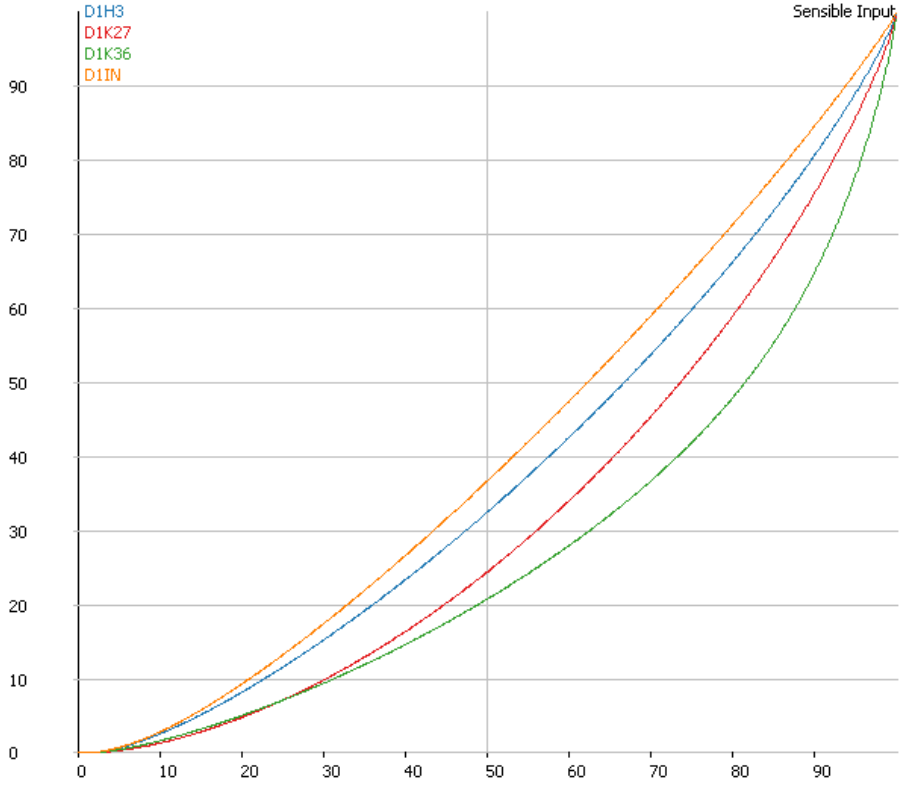
Compare samples

Summarise distributions



Cumulative Distribution Plot



QQ Plot

- Flatness of input
- Separation of ChIPs
- Consistency of ChIPs

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Associate enrichment with features

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Trend Plots

- Graphical way to look at overall enrichment relative to positions in features
  - Gene bodies
  - Promoters
  - CpG islands
- May influence how we later quantitate and analyse the data
  - Analyse per feature
  - Look for exceptions to the general rule

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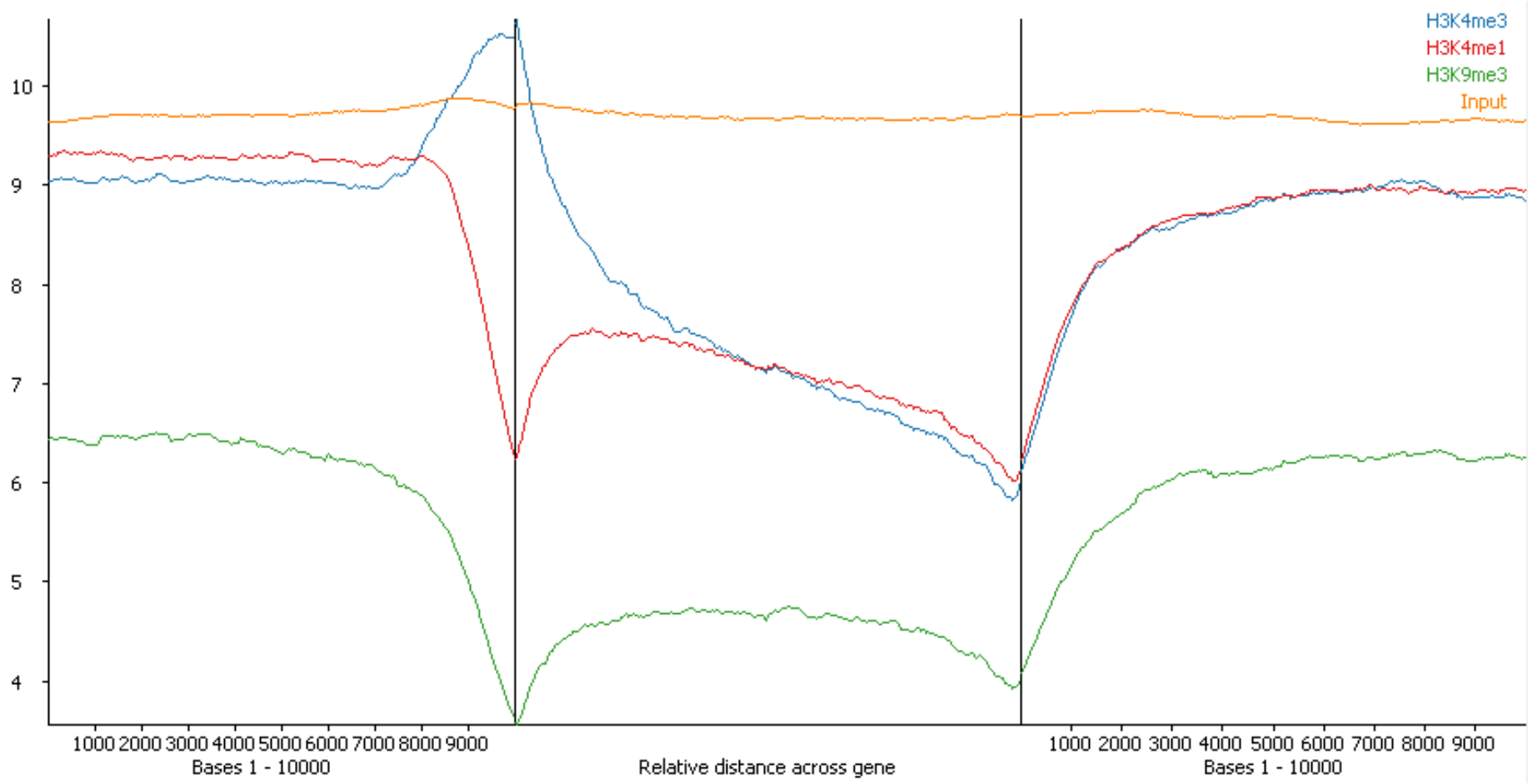
Trend Plot Example



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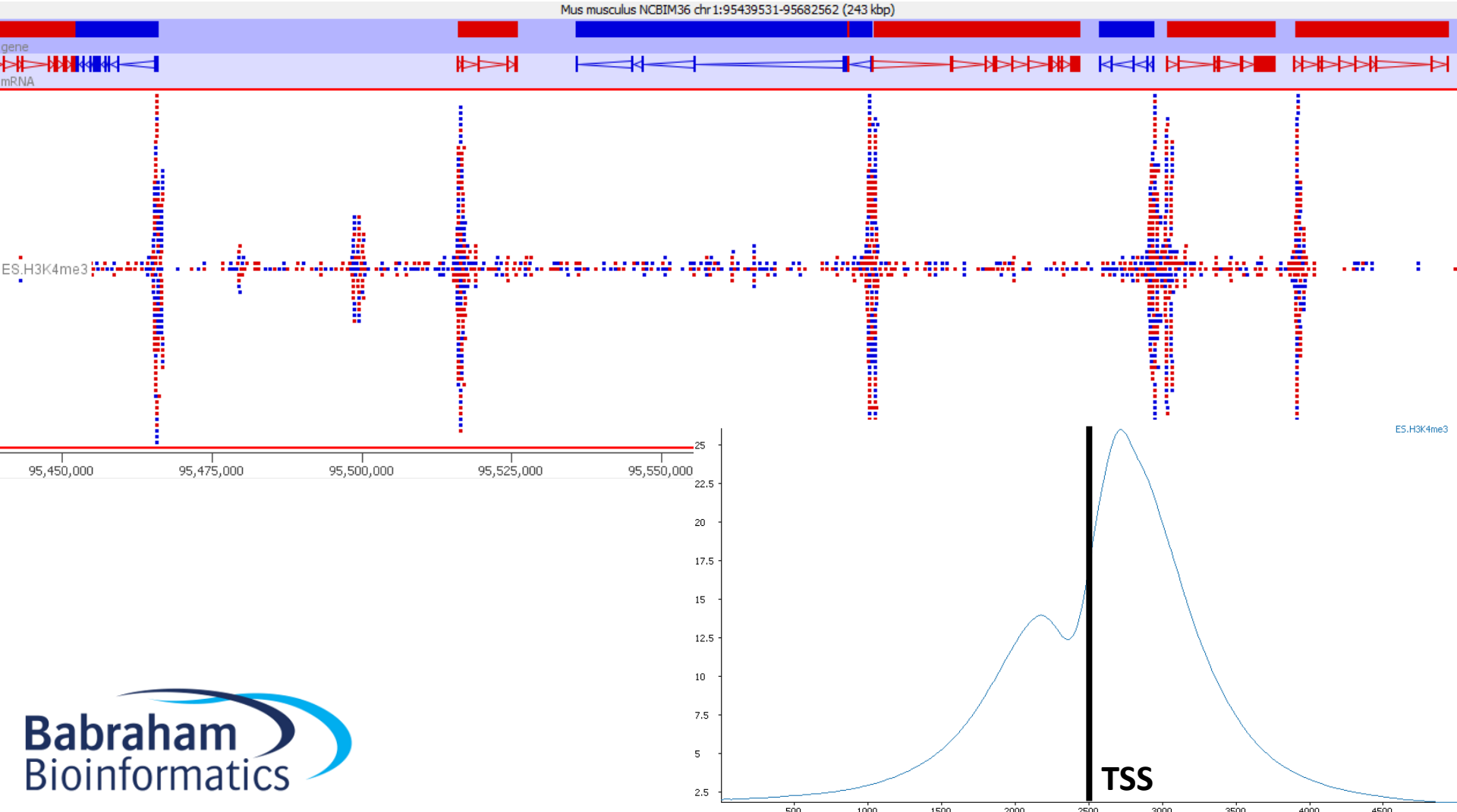
- Overall average
- Says nothing about proportion of features affected

Trend plots should match the data



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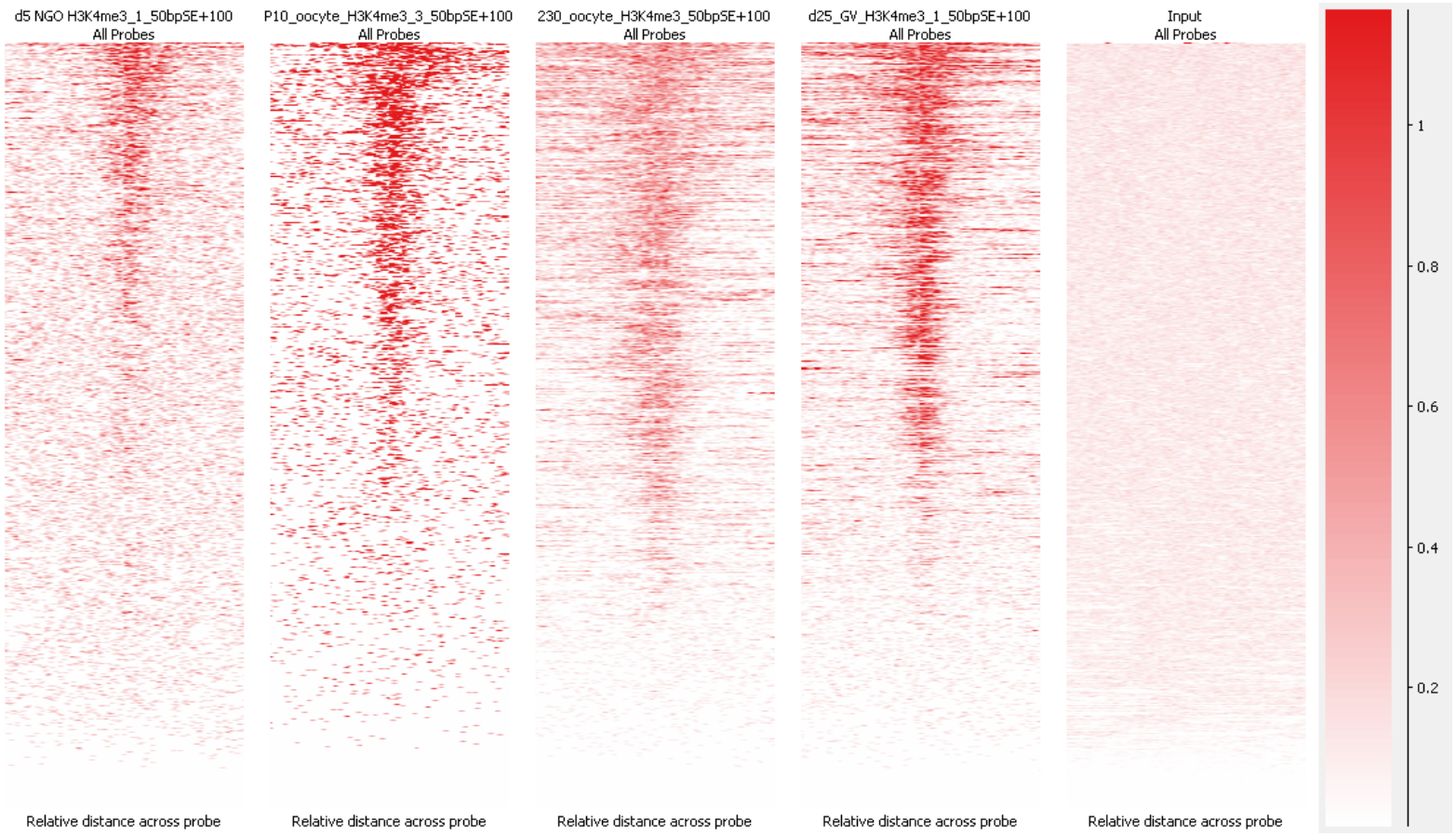
Aligned Probes Plots give more detail



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- Information per feature instance
- Comparison of equivalent features in different marks/samples

After exploration you should..

- Know whether your ChIP is really enriched
- Know the nature / shape of the enrichment
- Know whether your controls behave well
- Know whether you're likely to have differential enrichment
- Know if you will need additional normalisation
- Know the best strategy to measure your data

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