Visualising and exploring methylation data

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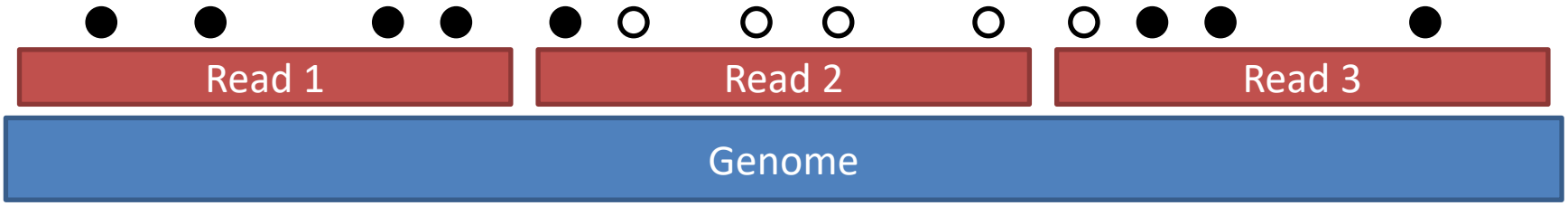
Employment disclosures:

Materials obtained from:

Starting Data




```
L001_bismark_bt2_pe.deduplicated.bam

CHG_OB_L001_bismark_bt2_pe.deduplicated.txt.gz
CHG_OT_L001_bismark_bt2_pe.deduplicated.txt.gz
CHH_OB_L001_bismark_bt2_pe.deduplicated.txt.gz
CHH_OT_L001_bismark_bt2_pe.deduplicated.txt.gz
CpG_OB_L001_bismark_bt2_pe.deduplicated.txt.gz
CpG_OT_L001_bismark_bt2_pe.deduplicated.txt.gz

L001_bismark_bt2_pe.deduplicated.cov.gz
```

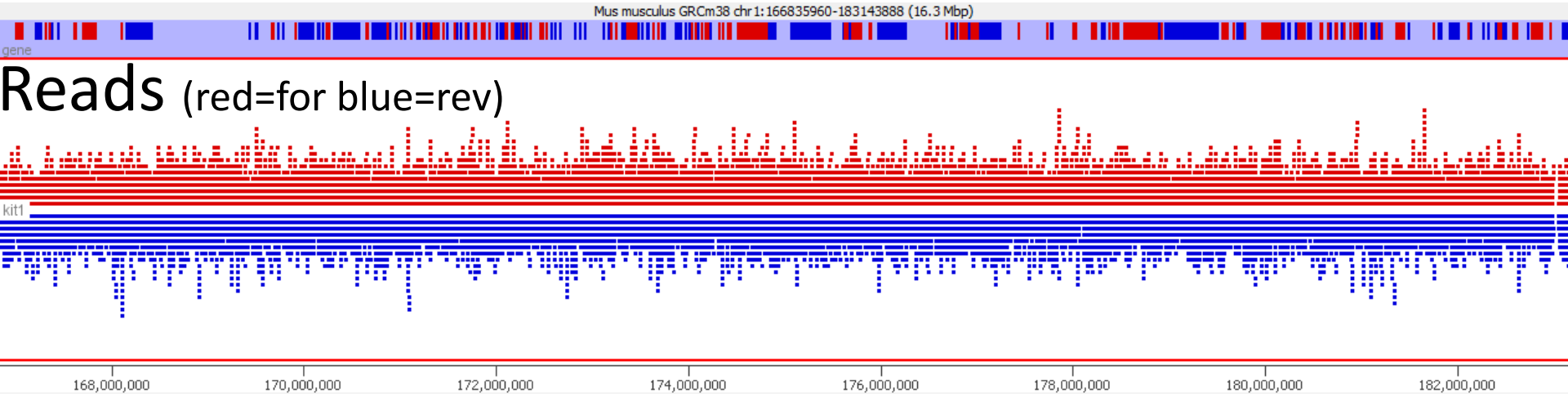
Decide early on which data to use

- Methylation contexts
  - CpG: Only generally relevant context for mammals
  - CHG: Only known to be relevant in plants
  - CHH: Generally unmethylated

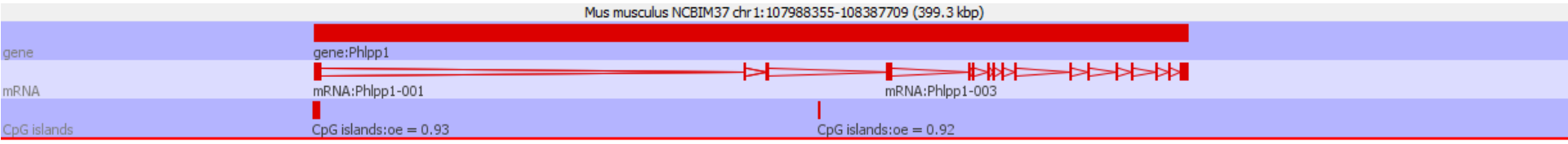
- Methylation strands
  - CpG methylation is generally symmetric
  - Normally makes sense to merge OT / OB strands

Always start by looking at your data.

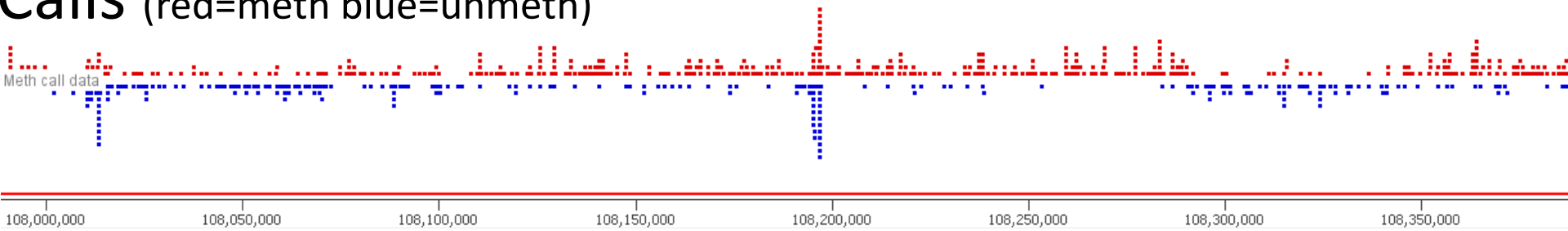
Think about what you expect



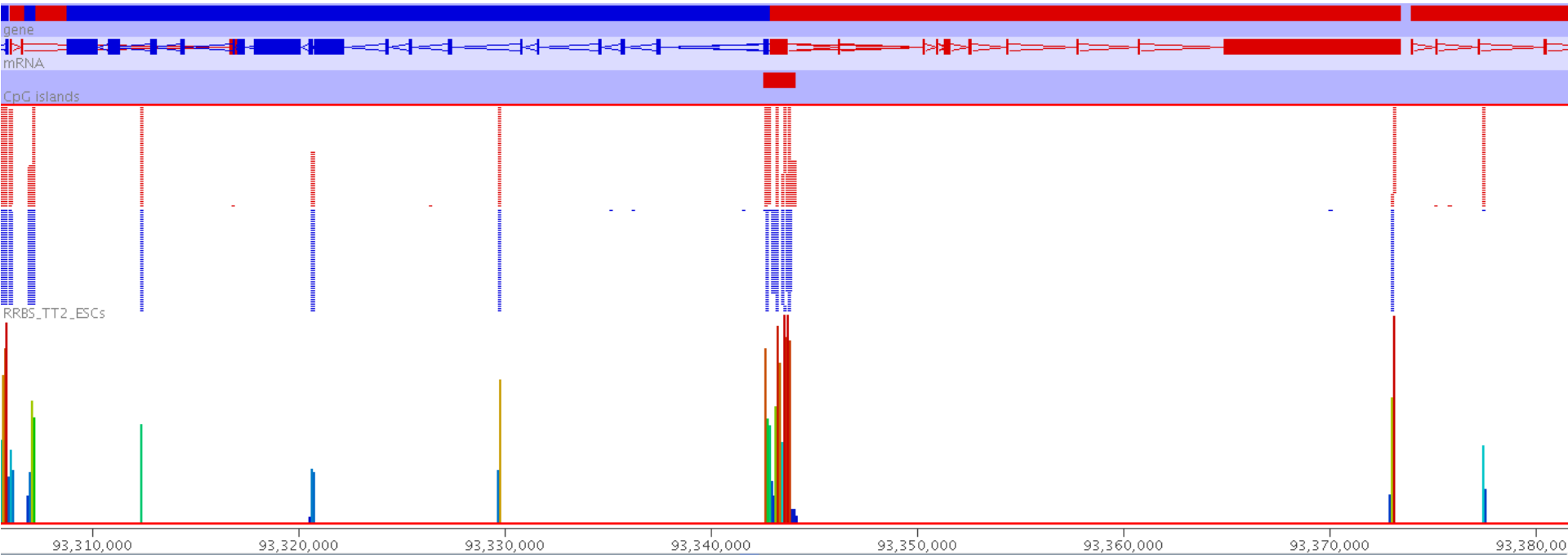
Reads (red=for blue=rev)



Calls (red=meth blue=unmeth)

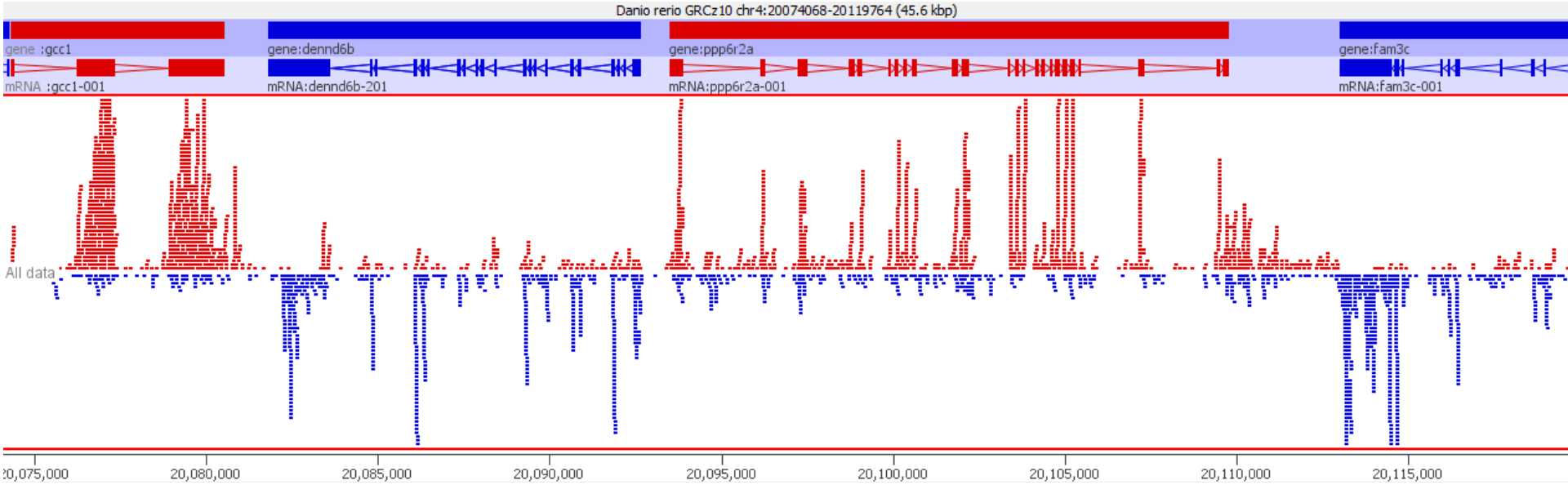


Try to understand anything unusual



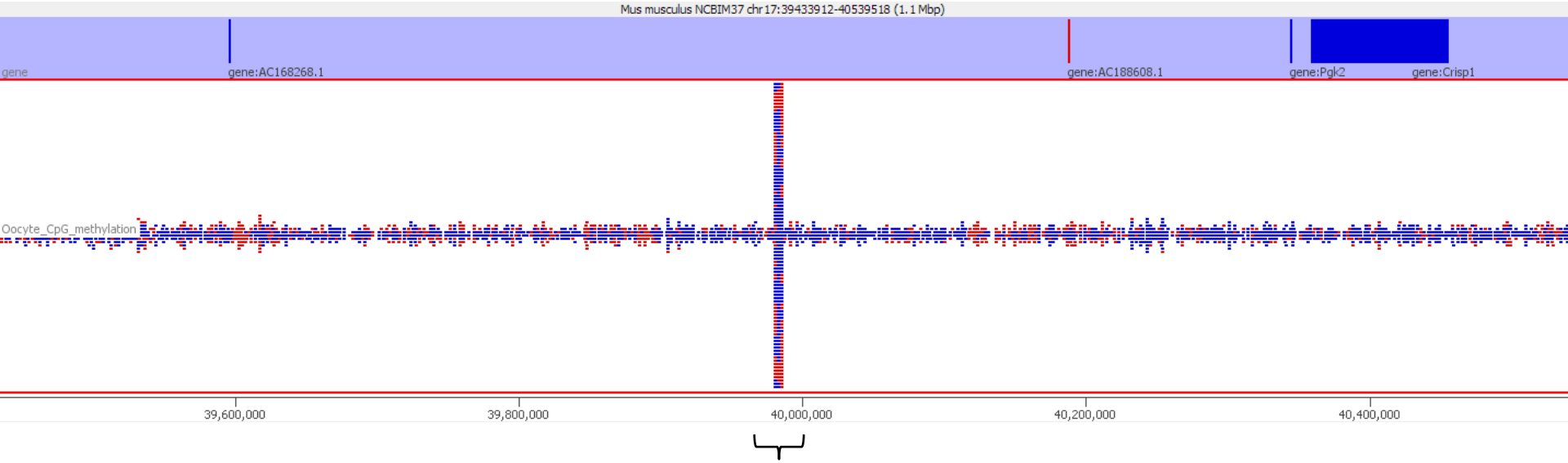
Reduced Representation Library

Try to understand anything unusual



Very messed up cDNA contaminated library

Try to understand anything unusual



Around 600x average genome density

Coverage Outliers

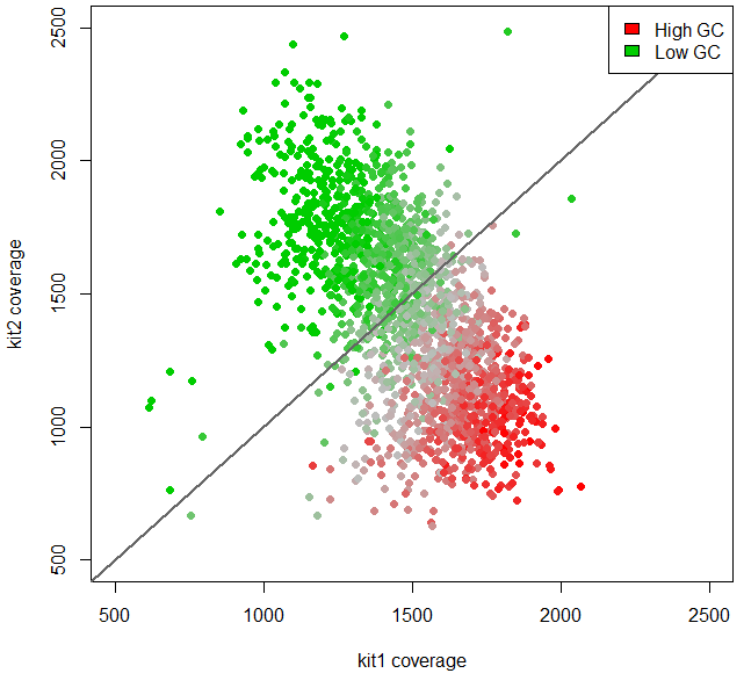
Coverage Outliers

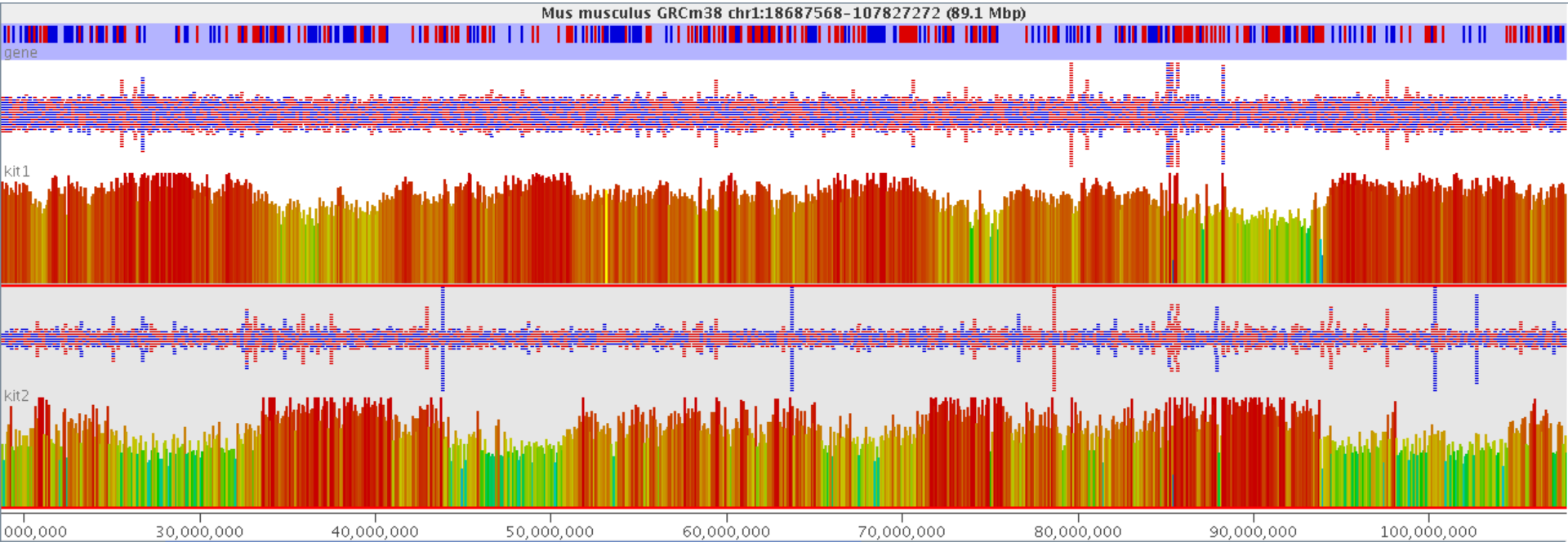
Coverage Outliers

- Normally the result of mis-mapping repetitive sequences not in the genome assembly
- Centromeric / telomeric sequences are common
- Can be a significant proportion of all data
- Can throw off calculations of overall methylation
- Should be flagged and hits in those regions ignored

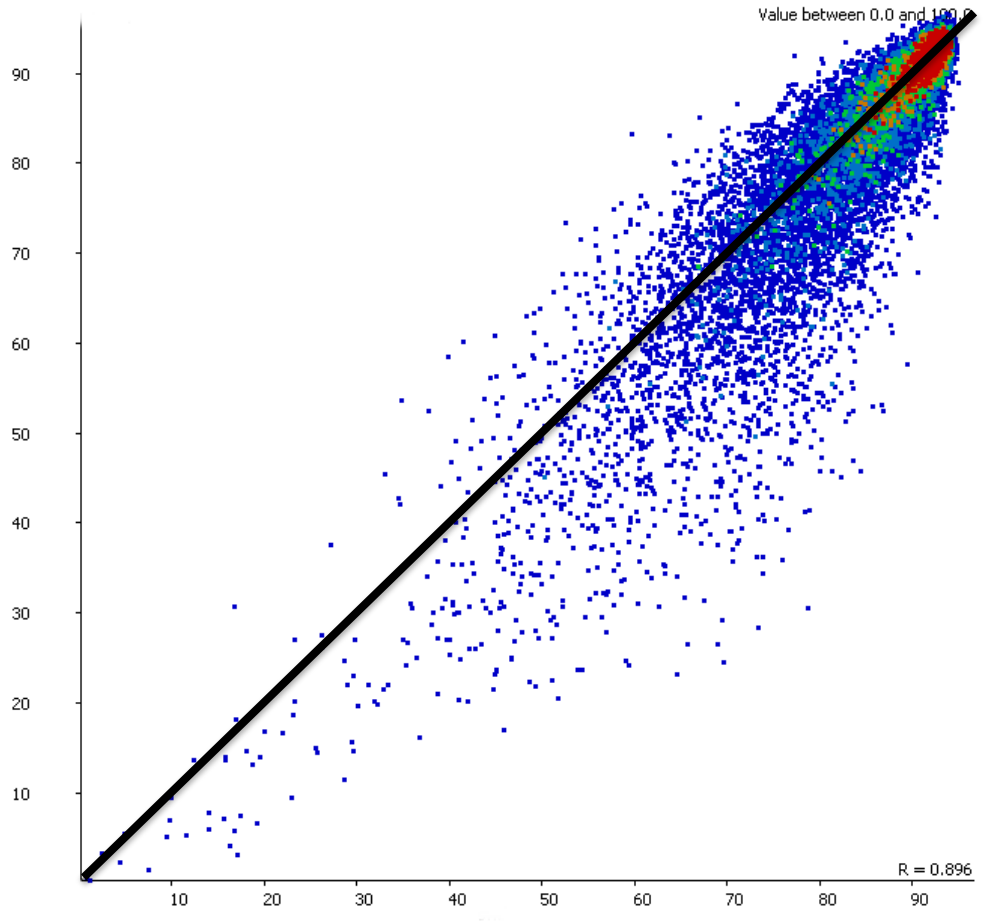
Coverage Bias

GC Content is most likely but others could exist





Coverage bias can lead to apparent methylation bias



Quantitating your methylation data

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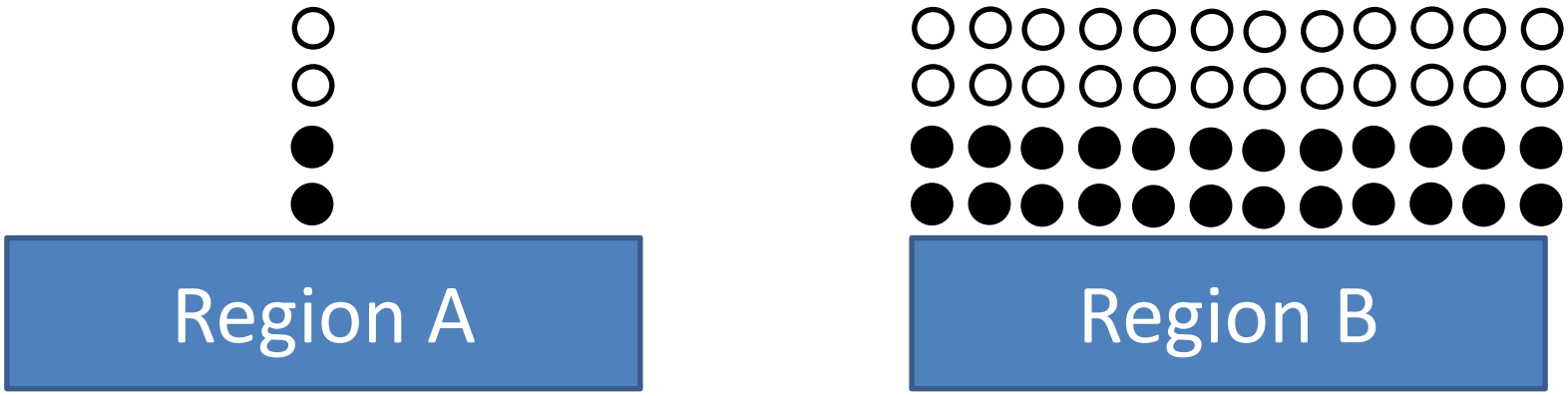
Where to make measures

- Per base
  - Very large number of measures
  - Poor accuracy for individual bases
- Unbiased windows
  - Tiled over whole genome
  - Need to decide how they will be defined
- Targeted regions
  - Which regions
  - What context

Accuracy and Power

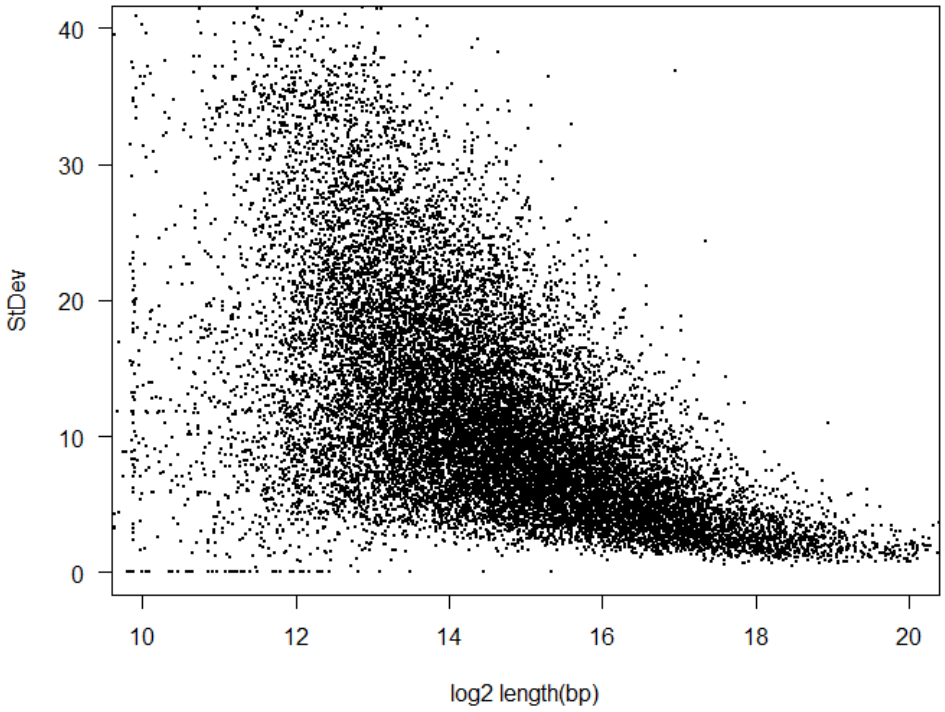


50% Methylation

50% Methylation

- Variation in CpG density
- Variation in coverage depth

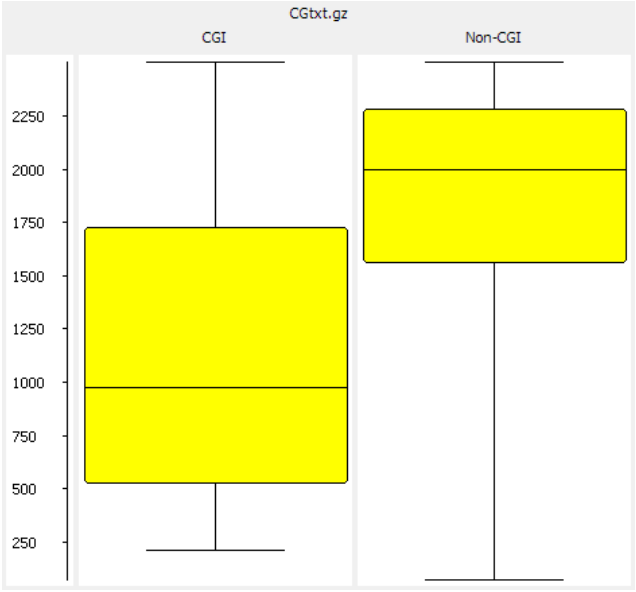
Try to make comparable measures



- Observation level correlates with stability.
- Want to try to have similar amounts of data in each measurement window.
- Equalises noise for visualisation and power for analysis.

Unbiased Analysis

- Fix the amount of data in each window
  - Fixed number of CpGs per window
  - Allow the resolution to vary



50 CpG window lengths

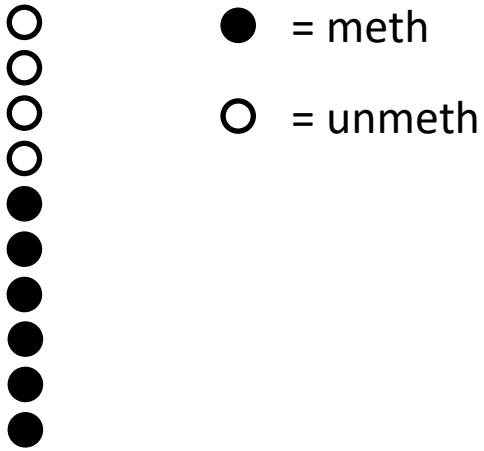
Targeted Quantitation

- Measure over features
  - CpG islands
    - Be careful where you get your locations
    - Try to fix sizes
  - Promoters
    - Should probably split into CpG island and non-CpG island
    - Try to fix sizes
  - Gene bodies
    - Filter by biotype to remove small RNA genes?

How to Quantitate methylation calls

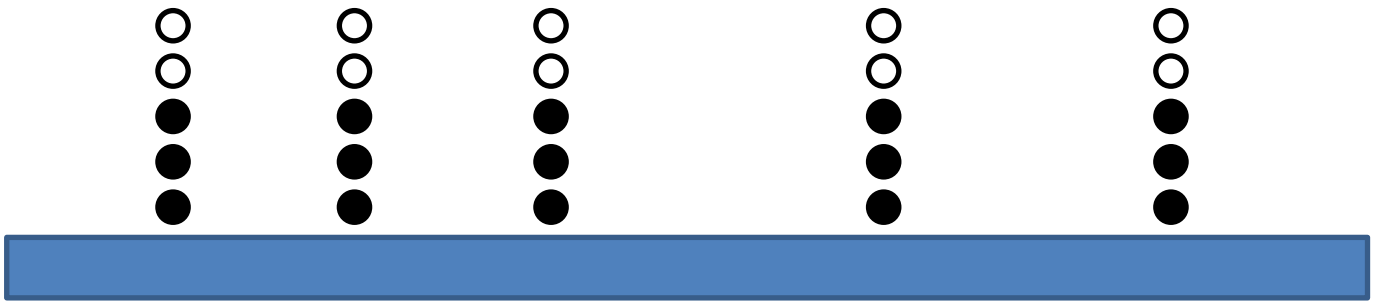
- Percentage methylation

  (Methylated calls / Total Calls) * 100



(6/10) * 100 = 60% methylated

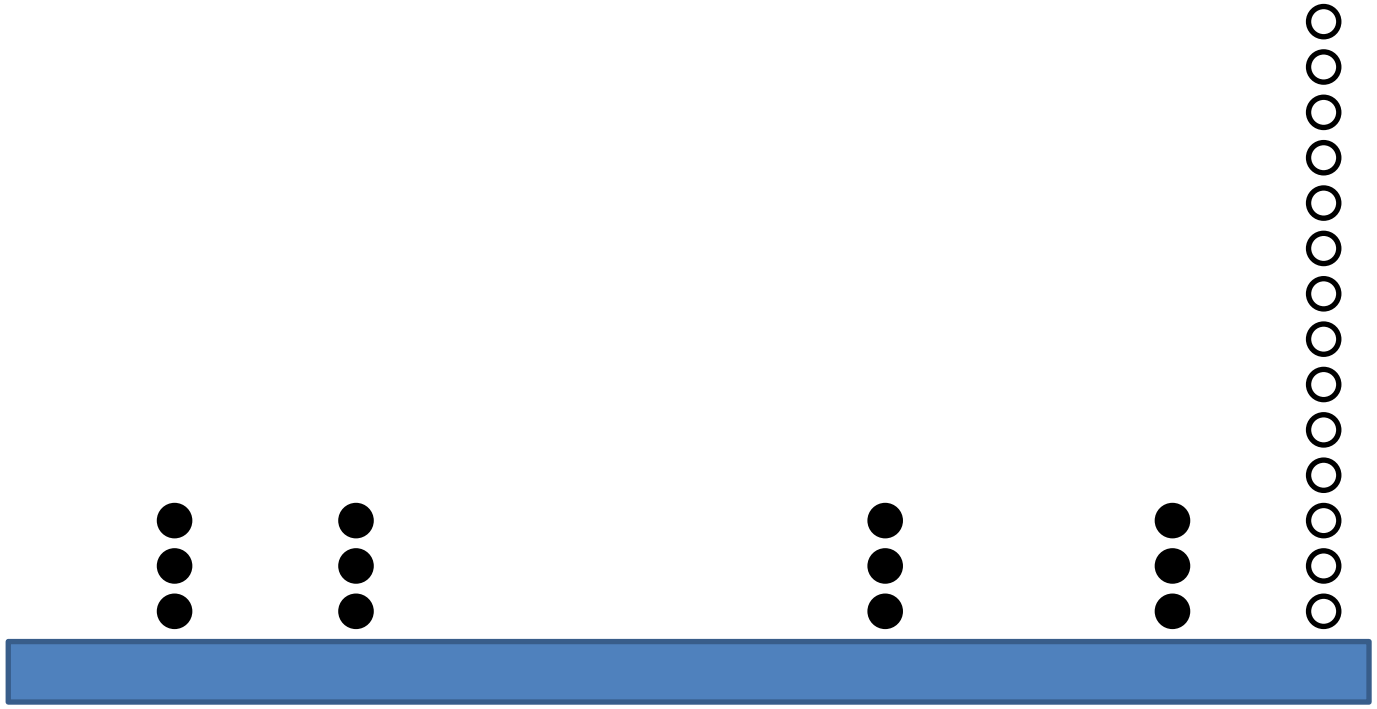
Assigning a % methylation value to a region can be difficult.



Total methylated calls = 15
Total unmethylated calls = 10

Methylation level = (15/(15+10))*100 = 60%

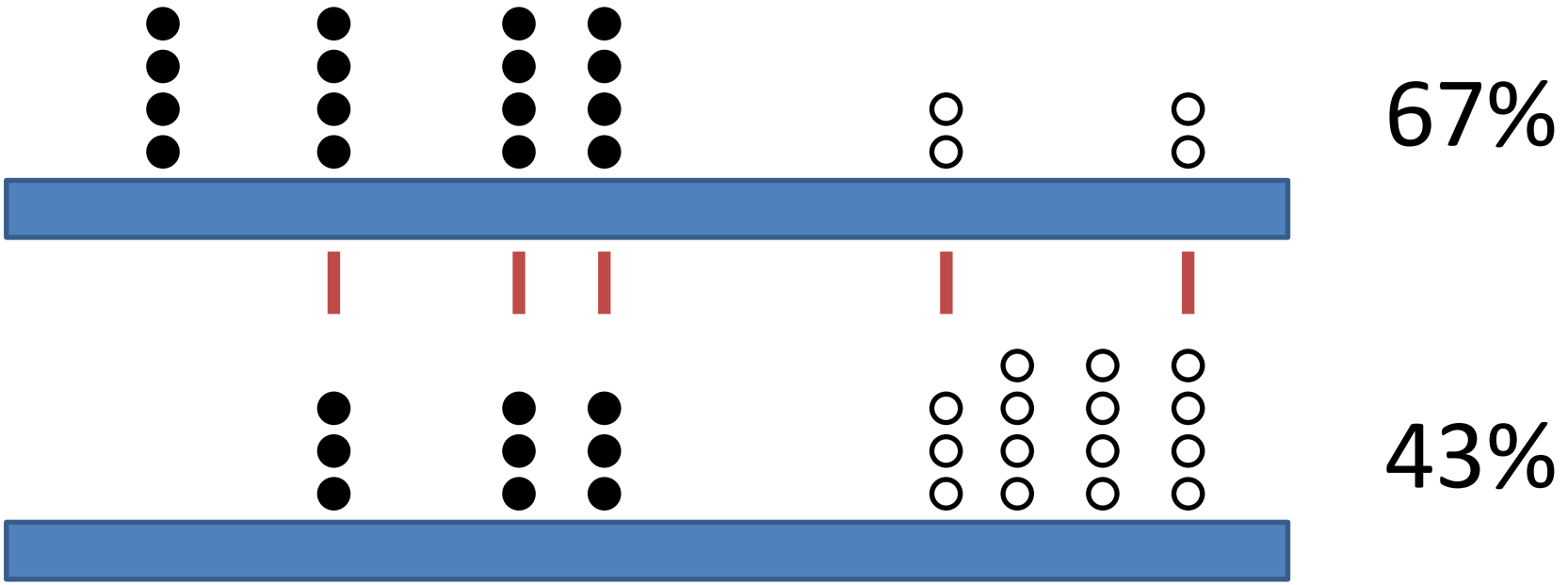
You get different answers quantitating per base or per region



Percentage methylation from all calls independently = 46%

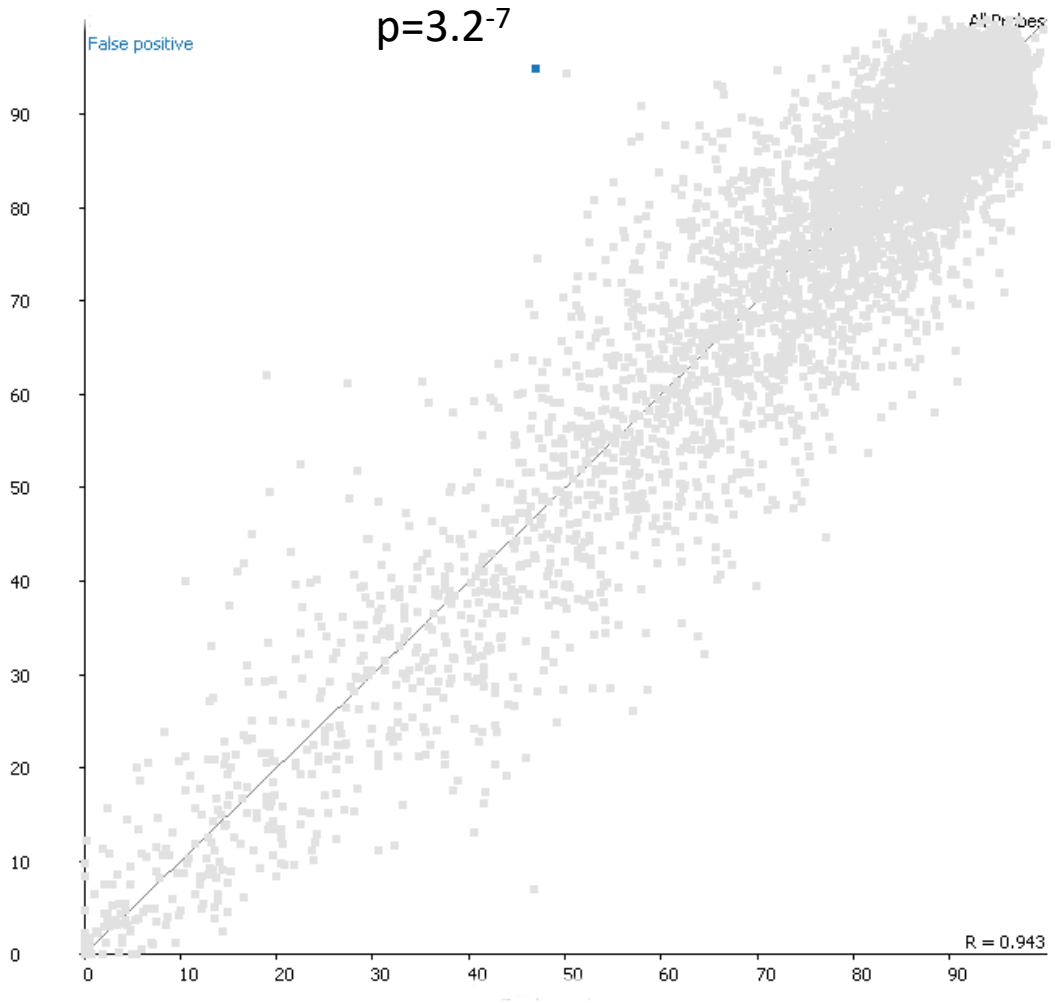
Percentage methylation from mean methylation per base = 80%

Coverage differences can look like methylation differences

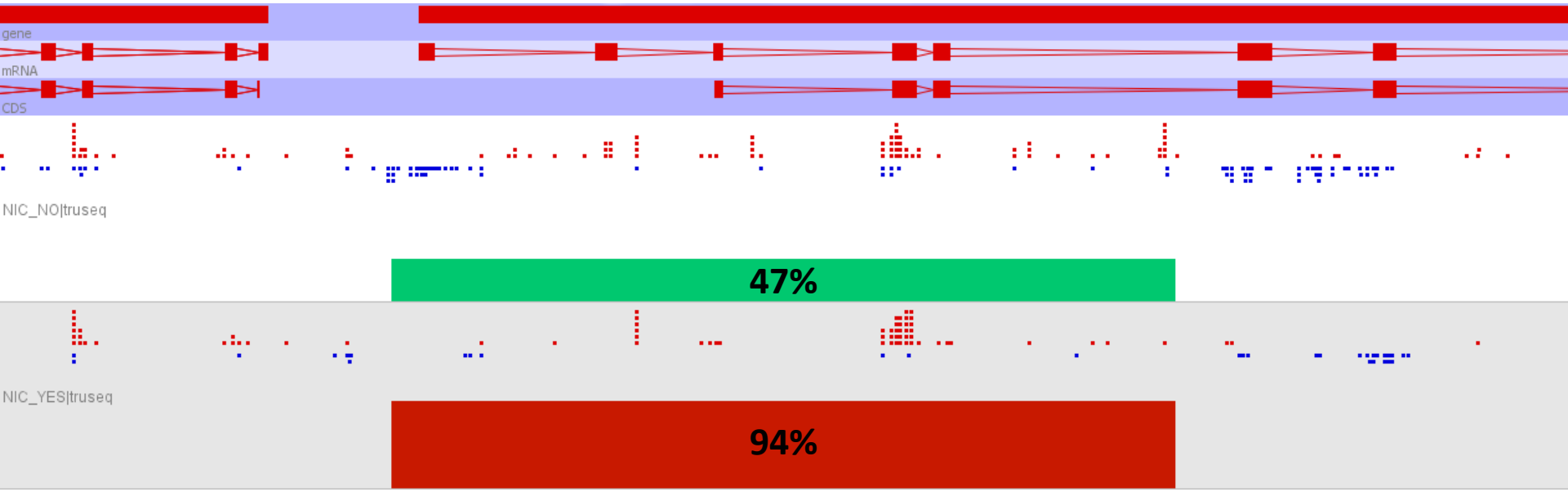


Common = 60% in both

Coverage differences aren't just a theoretical concern – they affect real data



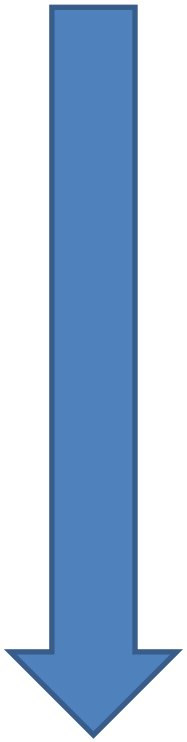
Coverage differences aren't just a theoretical concern – they affect real data



Levels of Complexity

- Percentage of all calls which are methylated
- Per base methylation, averaged over a region
  - Bases excluded because of low coverage
- As above, but requiring the same bases to be used in each sample
  - Doesn't scale well

Simple



Complex

(Even) More Complex Methods

- Smoothing or regression of actual measures along a chromosome.
  - Aims to reduce noise from sampling variation
  - Relies on consistent linear patterns
- Imputation of missing values
  - Relies on consistent linear patterns
- Additional normalisation or correction
  - Will be discussed later…

Visualisation and Exploration

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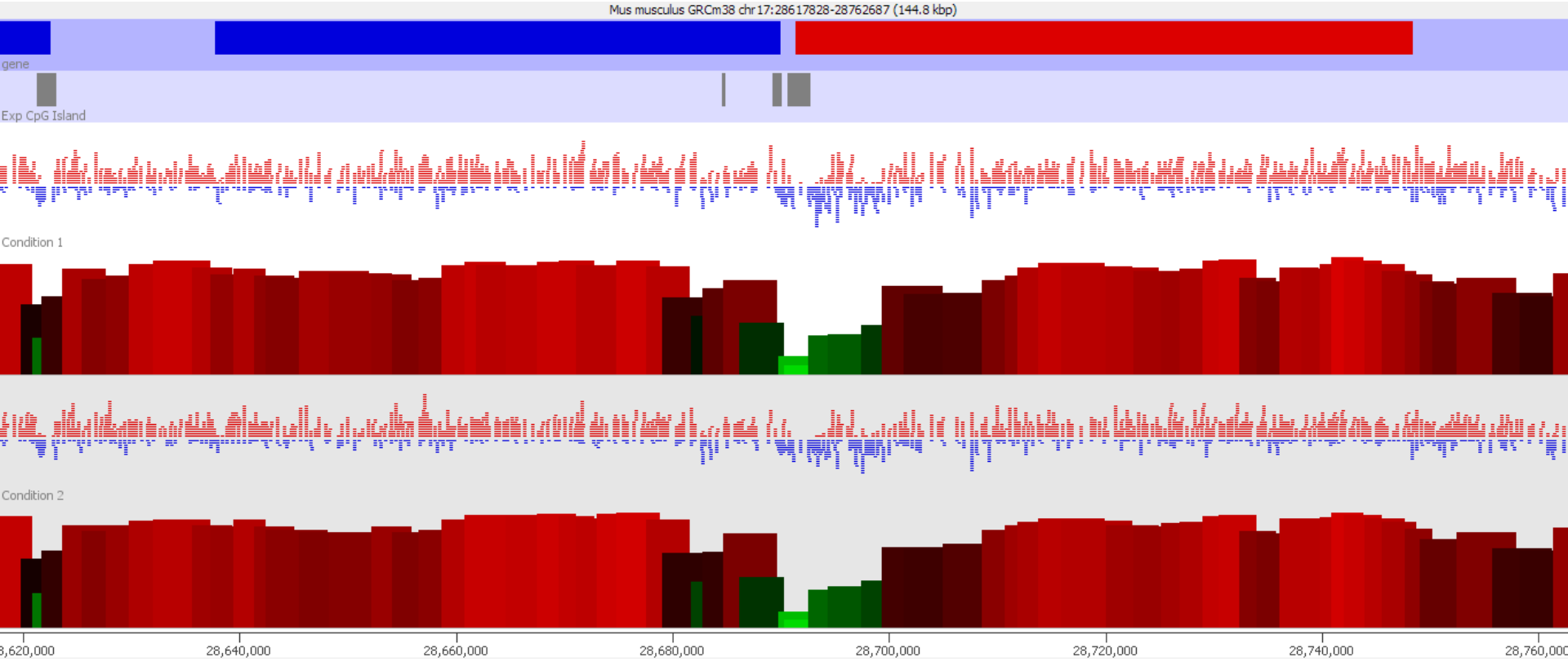
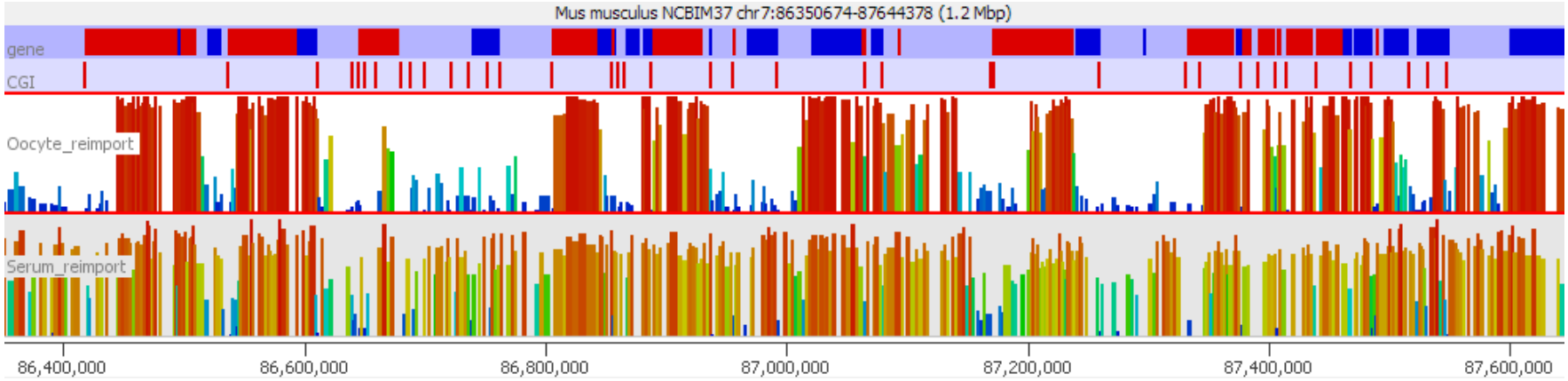
Use visualisation to understand the basic structure of your data before asking questions

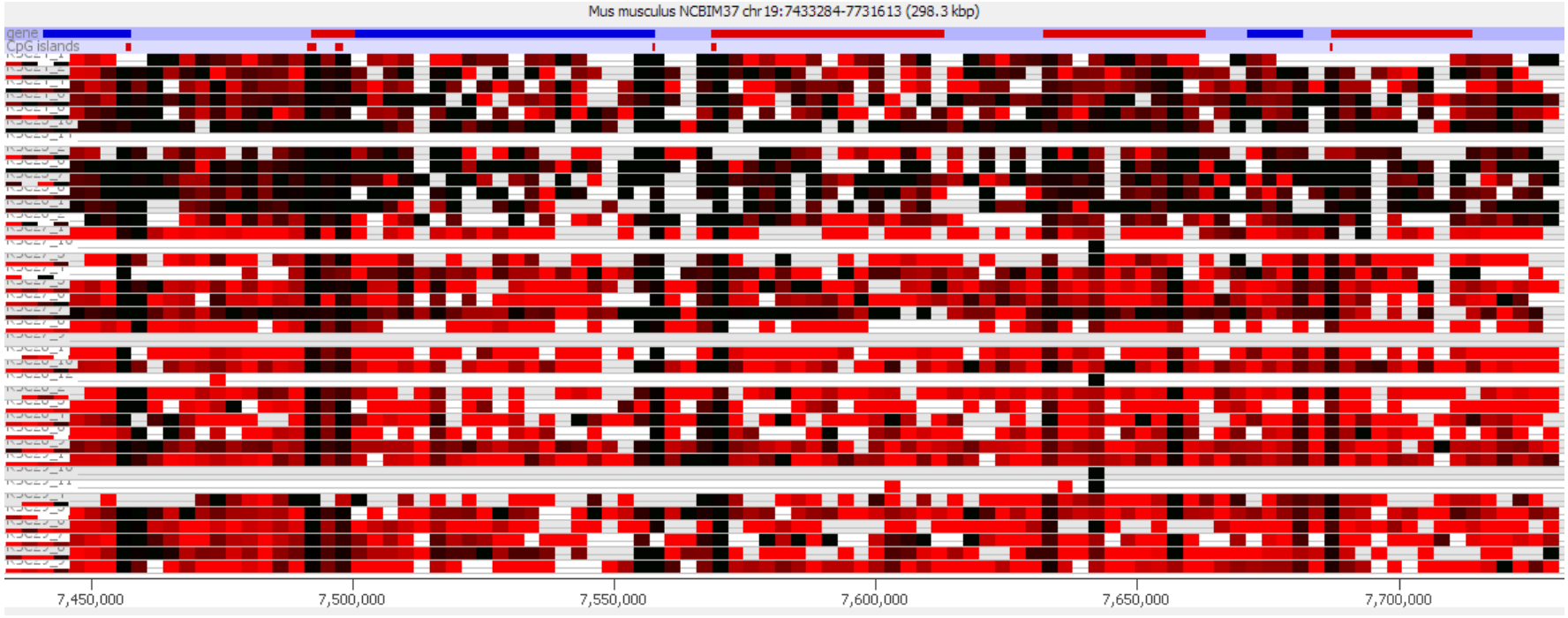
- Patterning
  - What sorts of changes in methylation do I observe along a chromosome
- Distributions
  - What are the overall levels and distributions of methylation values in my samples
- Relationships
  - On a global scale what is the overall relationship between methylation levels in different conditions

Visualise your quantitated data alongside the raw methylation calls.

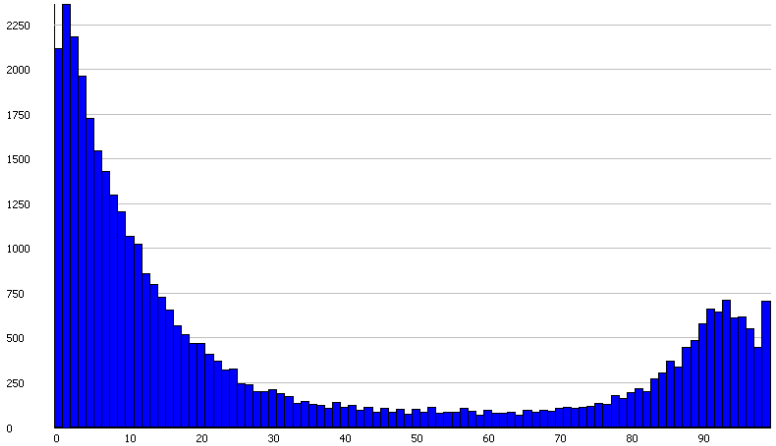


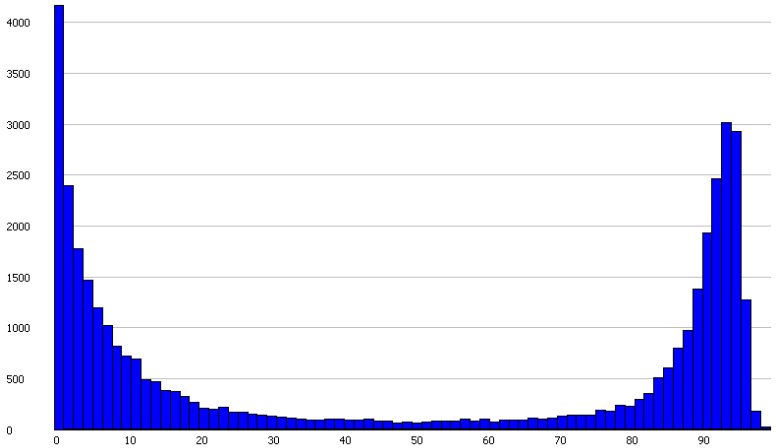
Different representations scale to different numbers of samples.

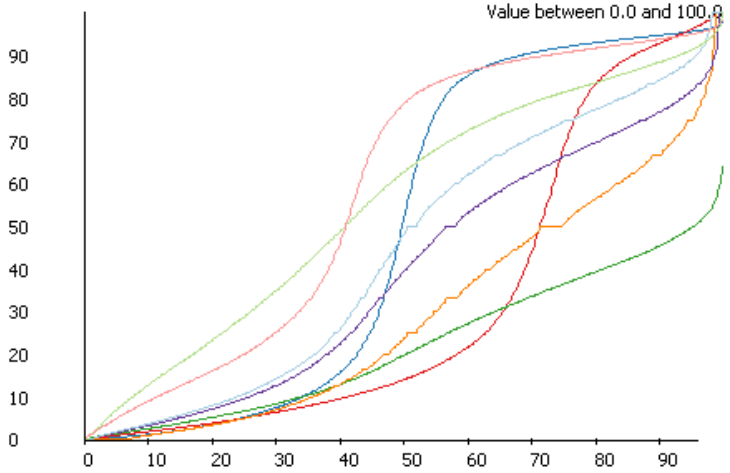


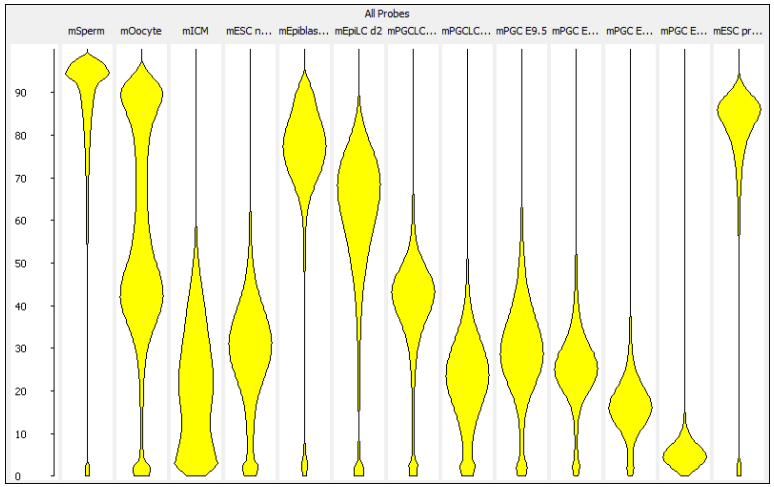


Understand and compare your methylation distributions before formulating a question.

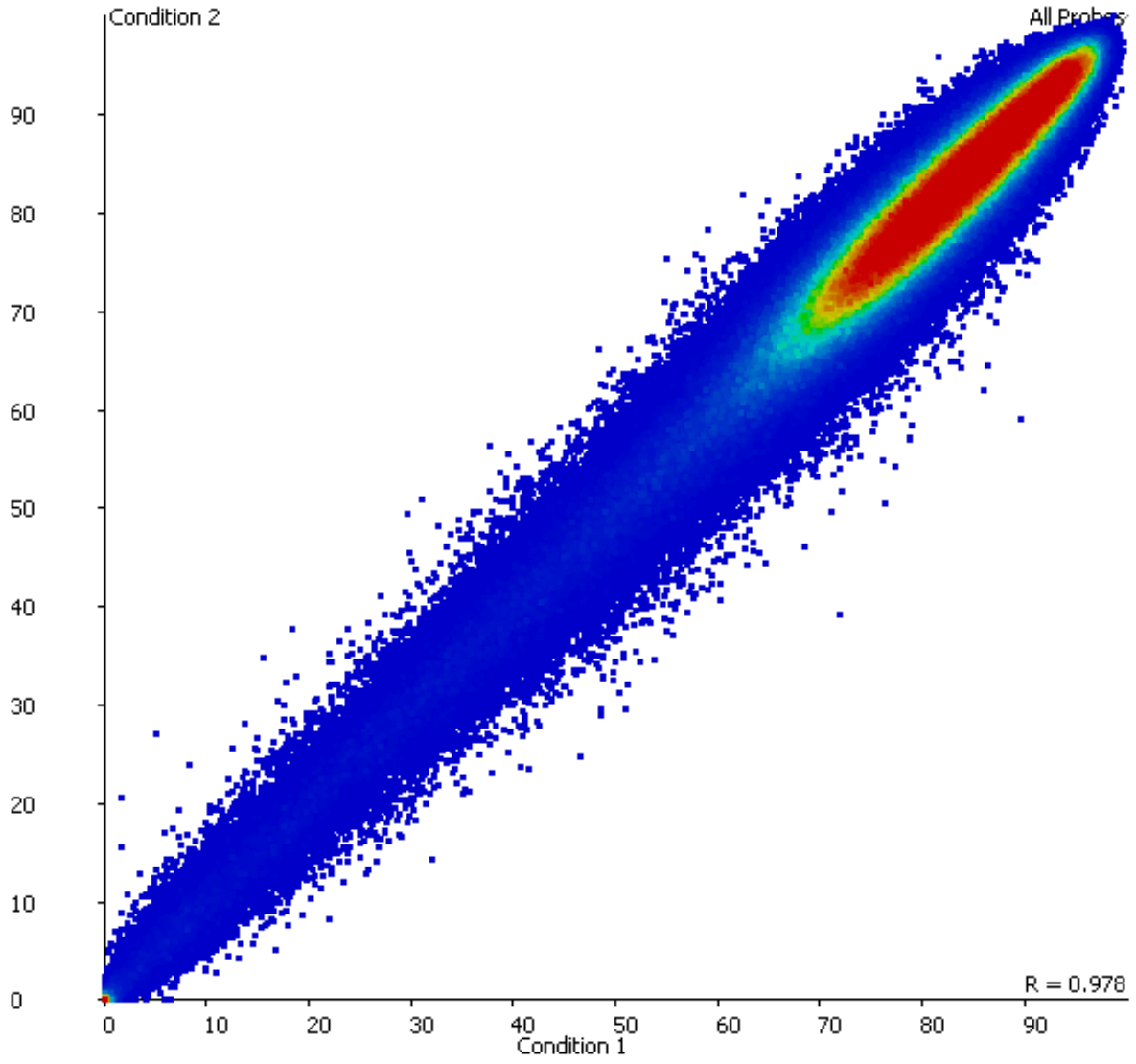




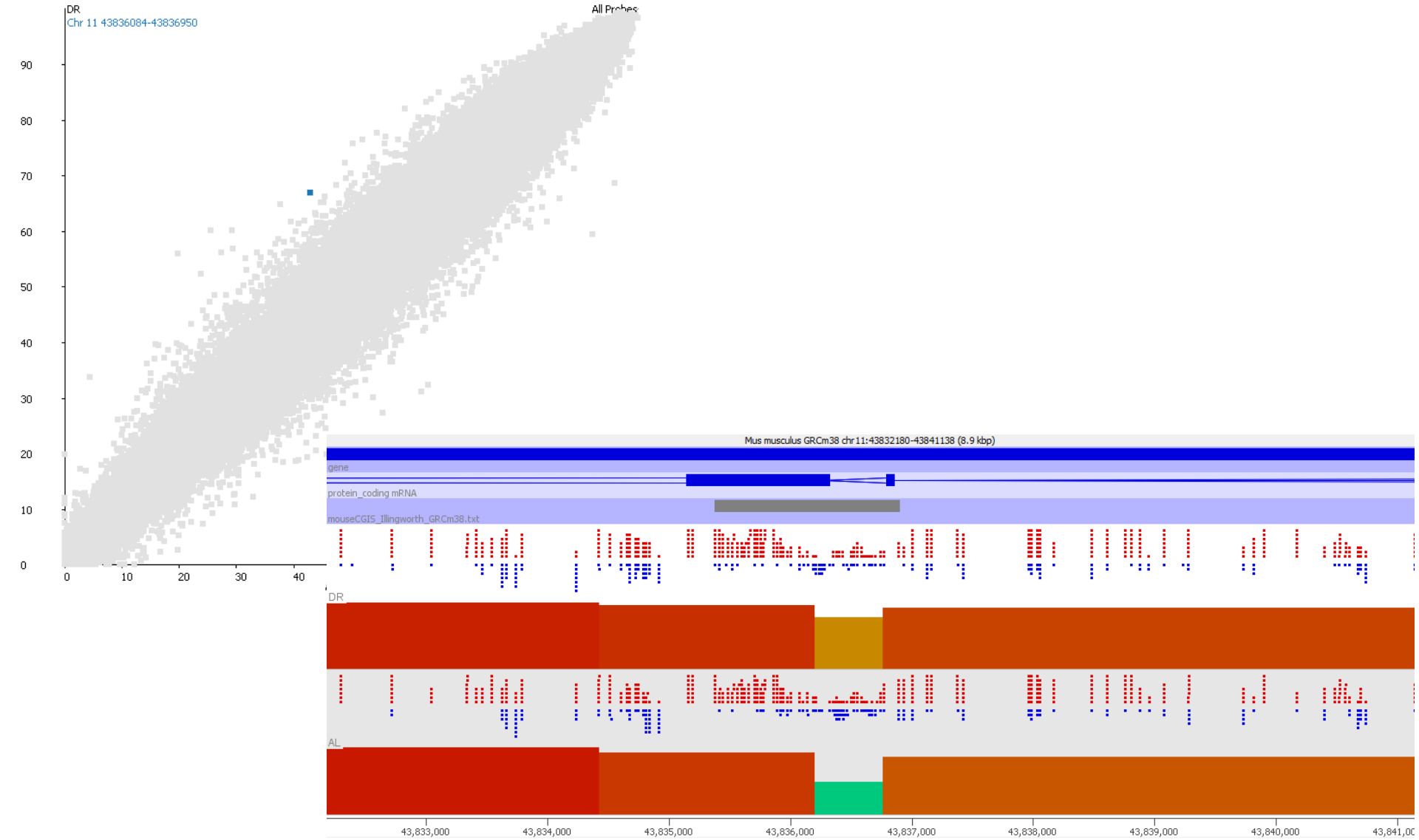




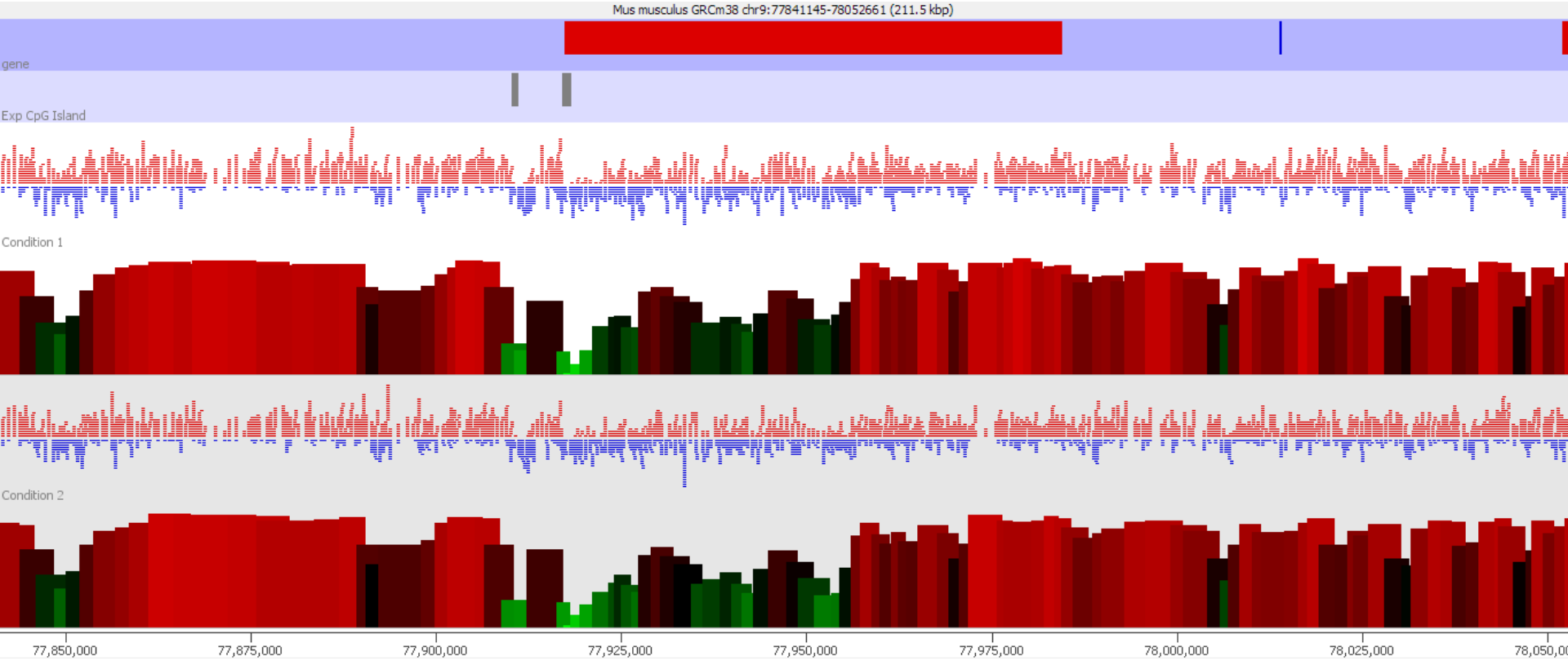
Plotting comparisons will identify global differences which might be interesting



Look at the data underneath and around potentially interesting points



Different representations might make the picture clearer



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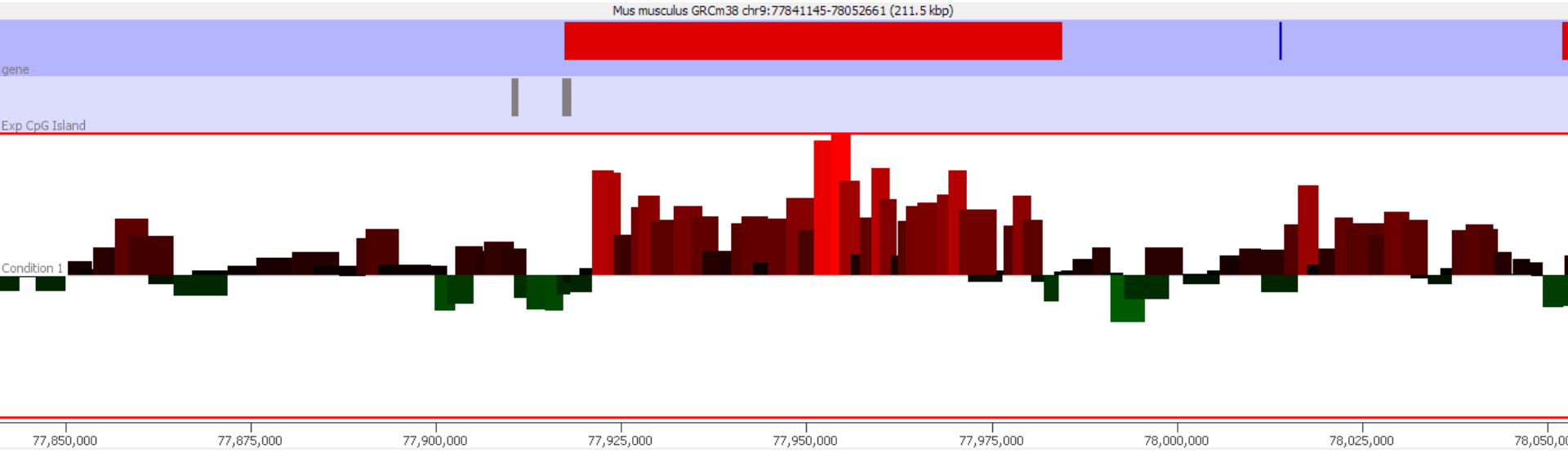
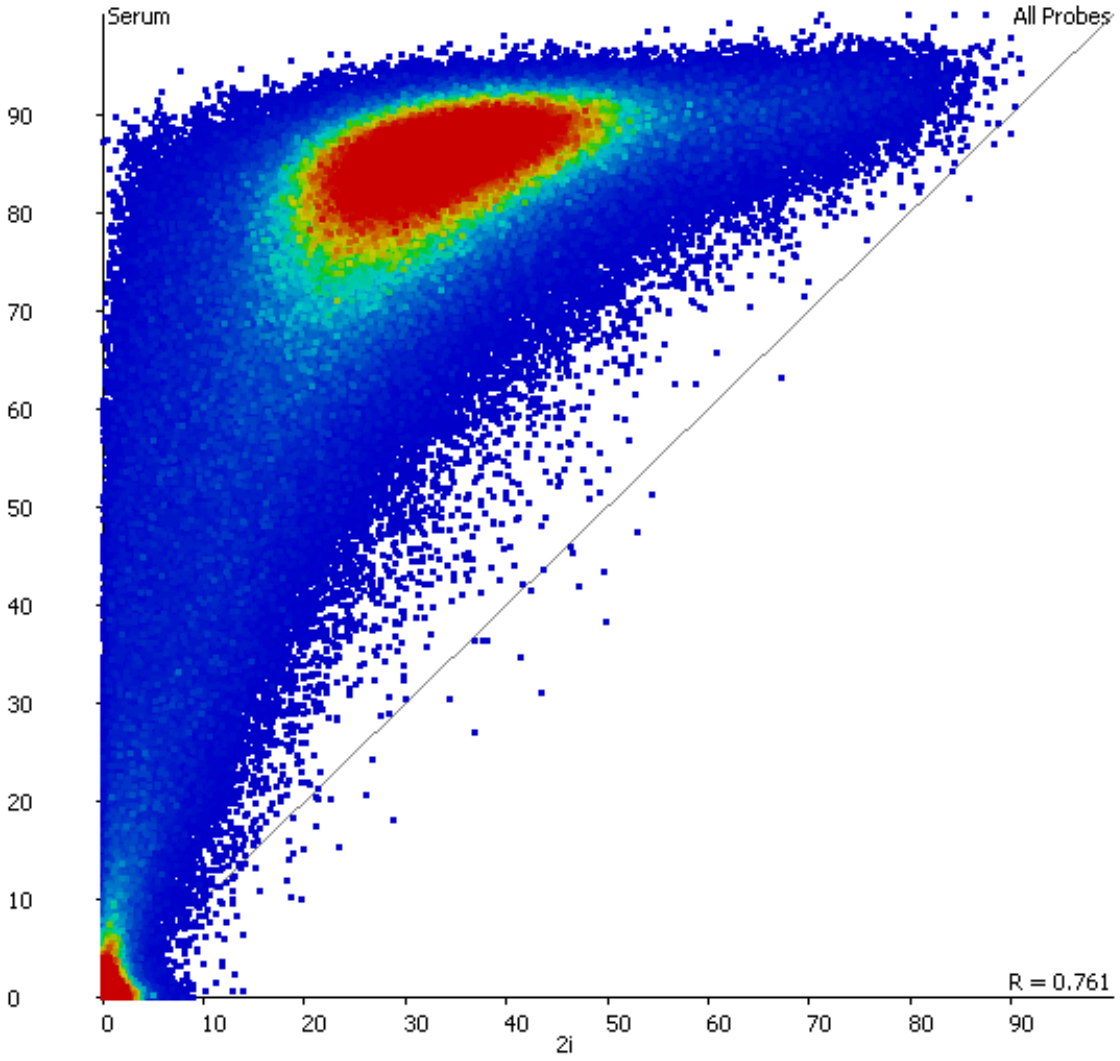
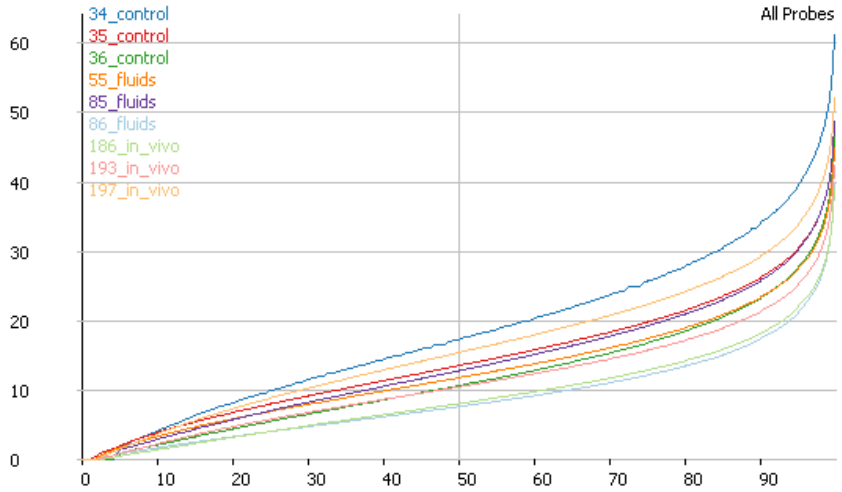
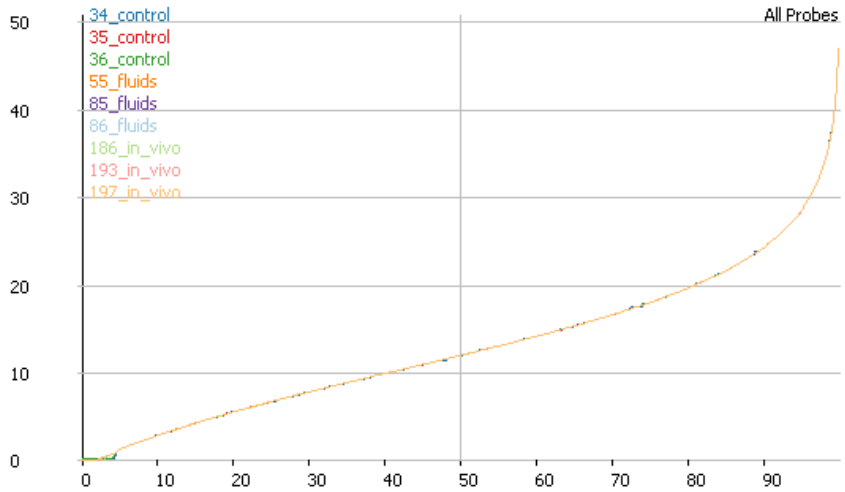


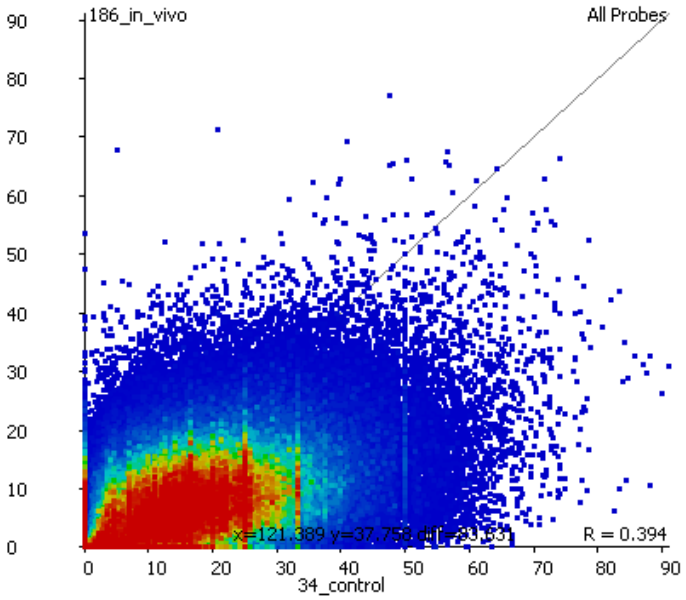
Large global changes might mean that local analysis is no longer relevant

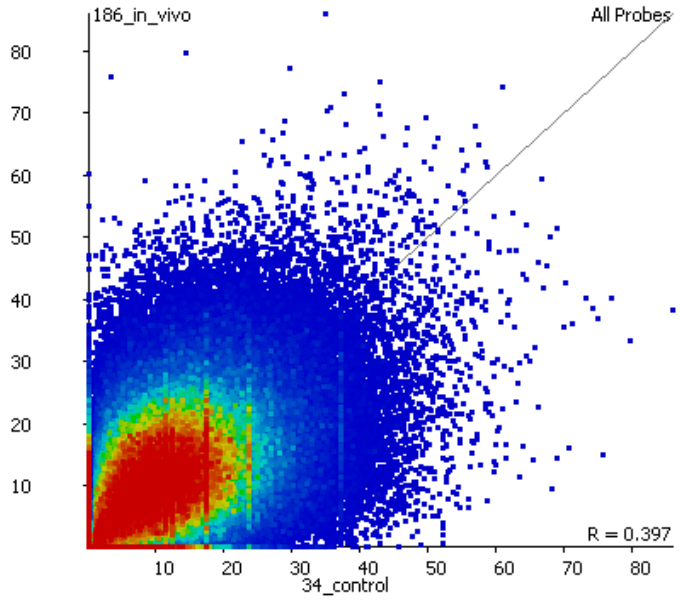


Small differences in distribution can be normalised to improve comparisons









Summary Visualisations

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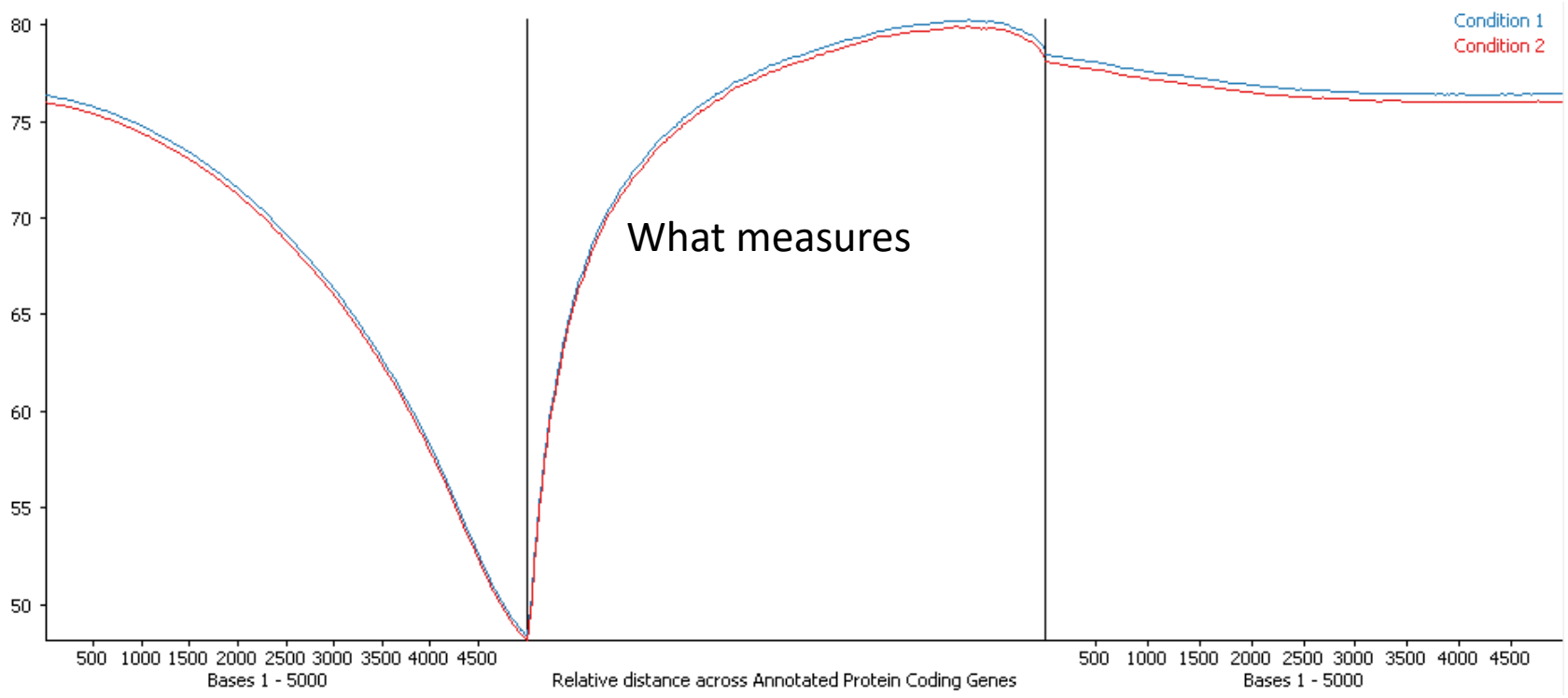
Trend Plots

- Effects at individual loci can be subtle
- Want to find more generalised effect
- Collate information across whole genome
- Look at the general trends
- Relies on the effect being consistent

Trend plot considerations

Axis Scaling



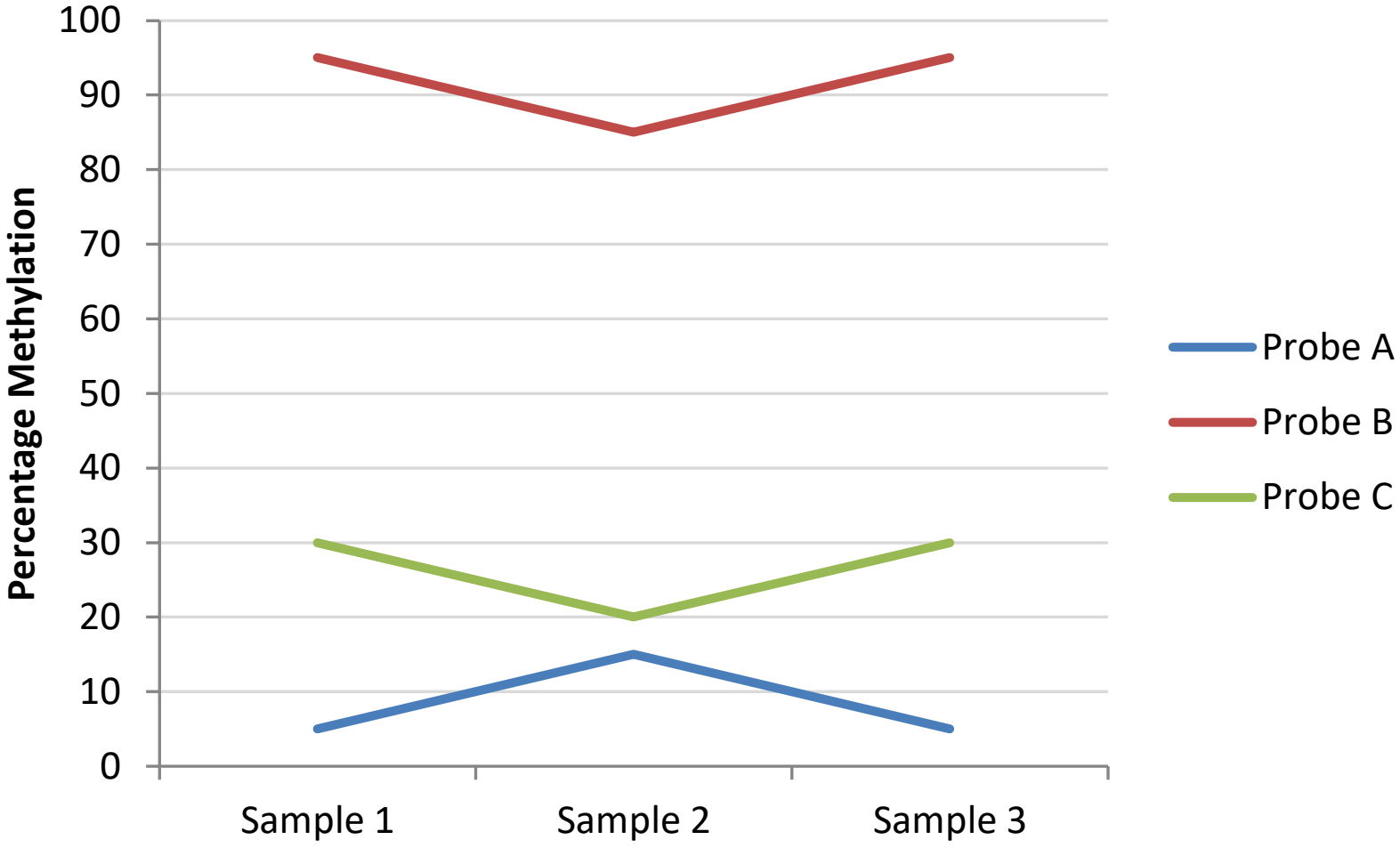
How much context

Features to use

How much context

Fixed vs relative scale

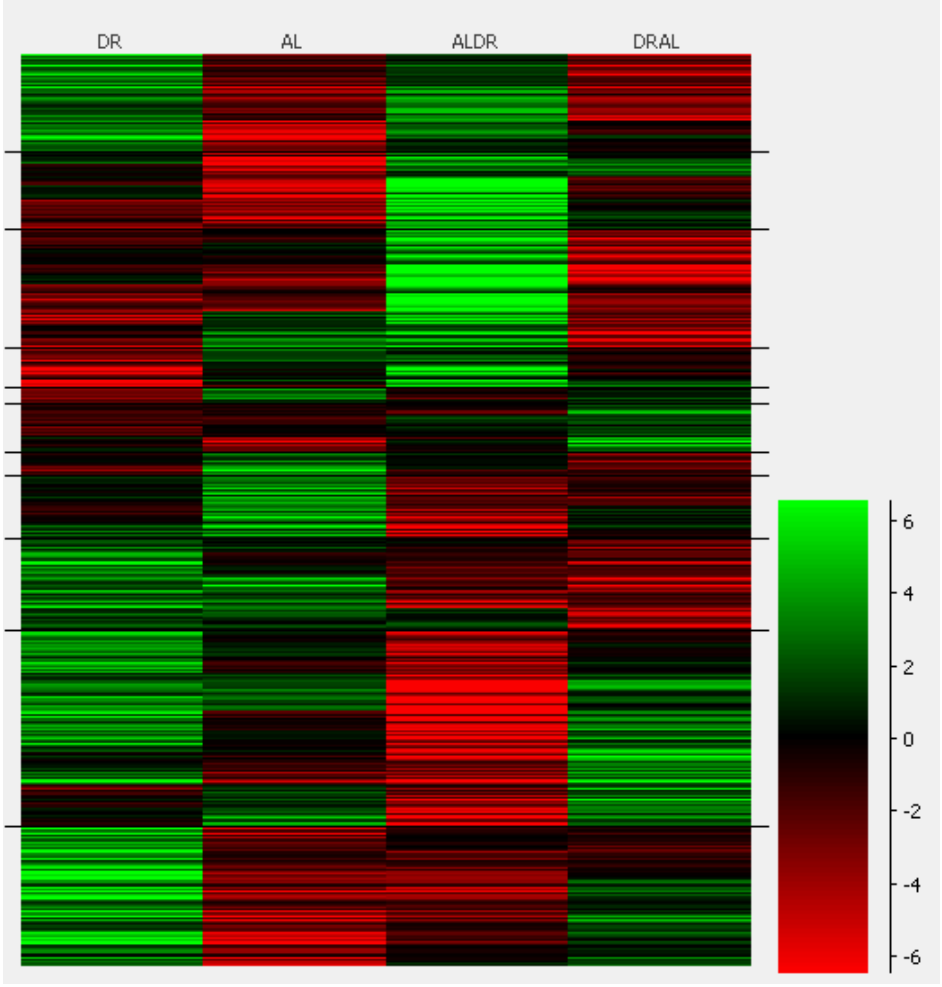
Clustering

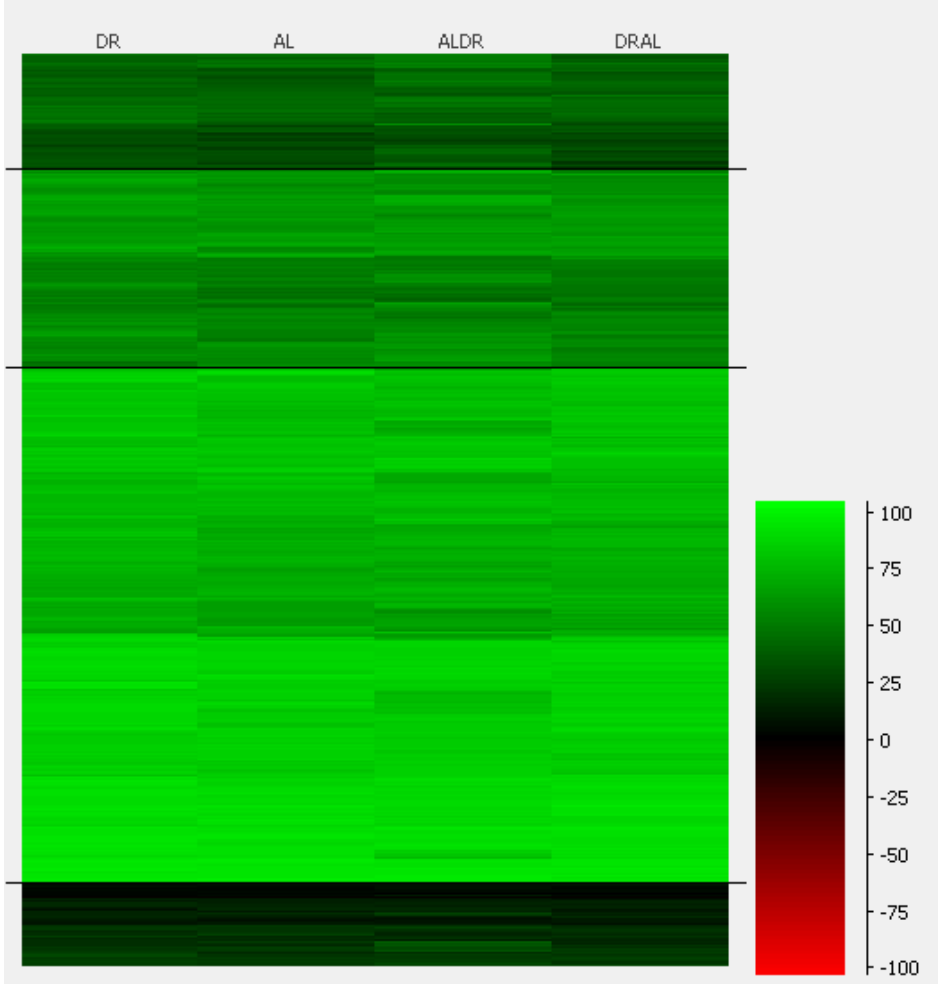

Percentage Methylation
100
90
80
70
60
50
40
30
20
10
0
Sample 1
Sample 2
Sample 3
Probe A
Probe B
Probe C

Clustering

- Correlation Clustering
  - Focusses on the differences between conditions
  - Absolute values not important
  - Look for similar trends
  - Show median normalised values
- Euclidean Clustering
  - Focusses on absolute differences between conditions
  - Look for similar levels
  - Show raw values

Clustering


DR
AL
ALDR
DRAL
6
4
2
0
-2
-4
-6


DR
AL
ALDR
DRAL
100
75
50
25
0
-25
-50
-75
-100

Exploration Summary (1)

- Look at the distribution of your raw reads/calls
  - Match expectations to the type of library
- Always start with an unbiased quantitation
  - Fix the amount of data in each window
  - Think about how to best quantitate
- Check the quantitation matches the raw data

Exploration Summary (2)

- Check the distributions of methylation values in your samples
- Directly compare your values to look for global differences
  - They might be the source of the interesting biology
  - Might spot small global differences which require normalisation
- Summarise trends around features
  - Might justify targeted quantitation