Visualising and Exploring BS-Seq Data

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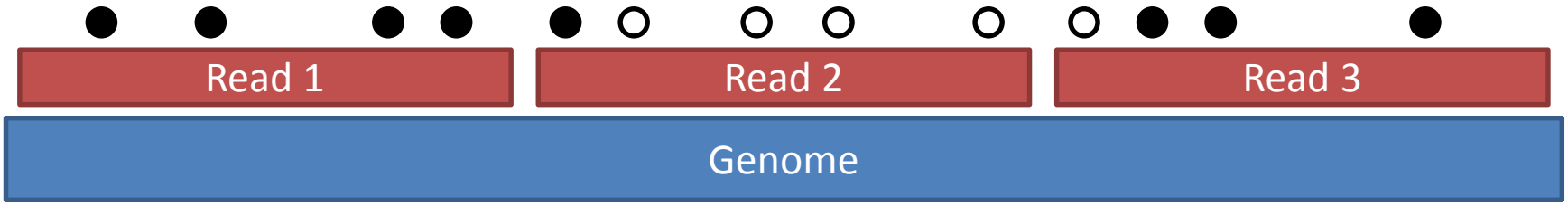
Starting Data




```
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
```

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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

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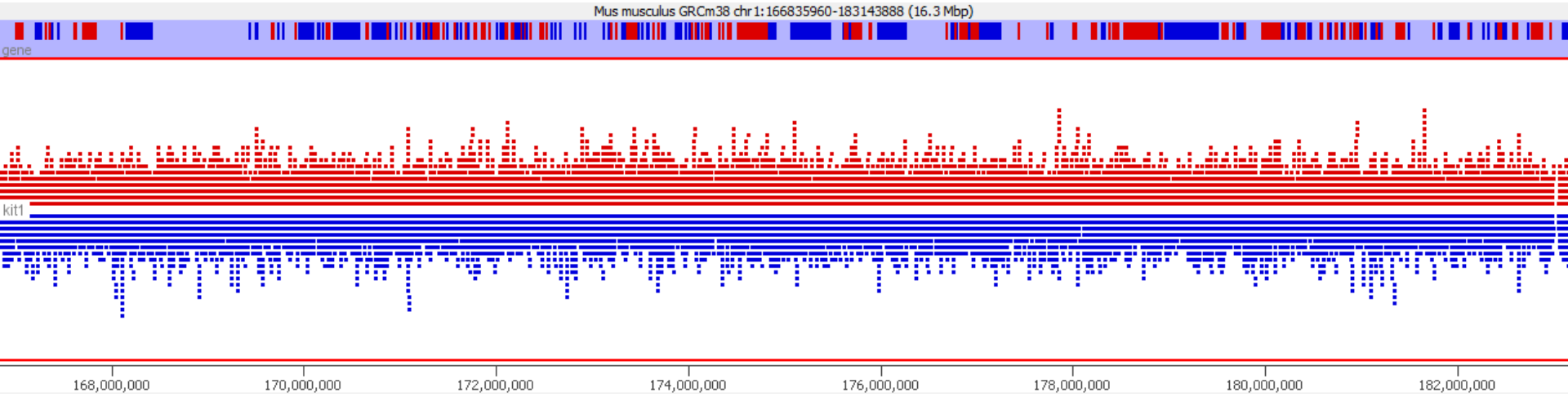
Coverage Expectations

- Whole genome covered equally
- Both strands present equally



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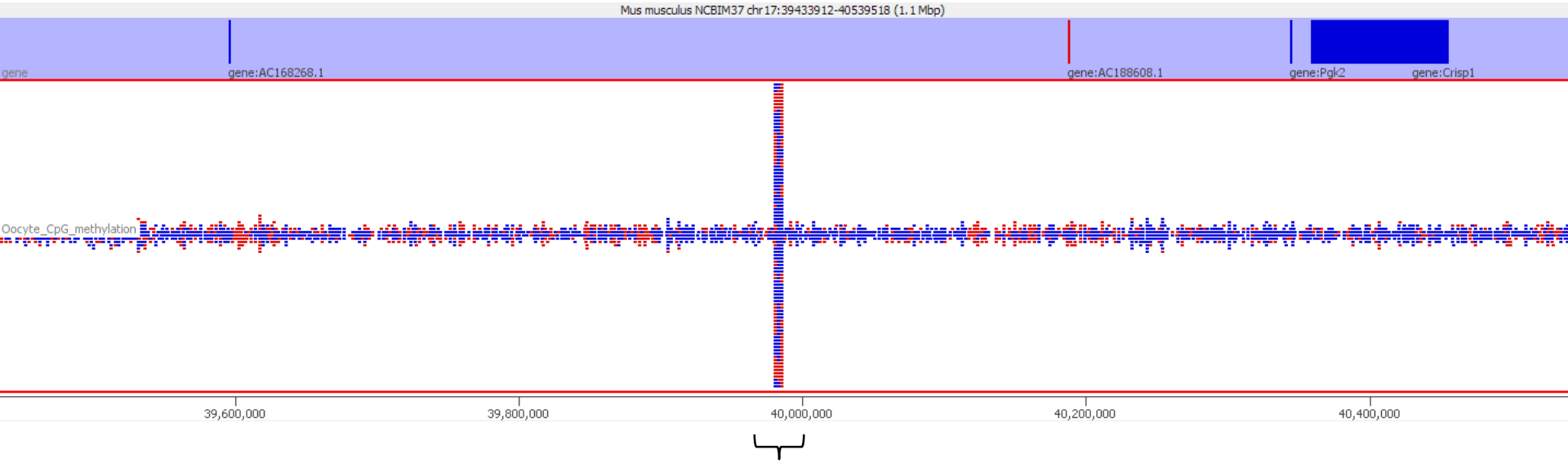
Coverage Outliers



Around 600x average genome density

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Coverage Outliers

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Coverage Outliers

- Normally the result of mis-mapping repetitive sequences not in the genome assembly
- Centromeric / temomeric sequences are common
- Can be a significant proportion of all data
- Can throw off calculations of overall methylation
- Should be removed

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Coverage Biases

- BS-Seq read level coverage should be flat
- Different kits have different biases
- Most bias is based on composition (normally GC)
- Link between methylation and GC content can cause problems.

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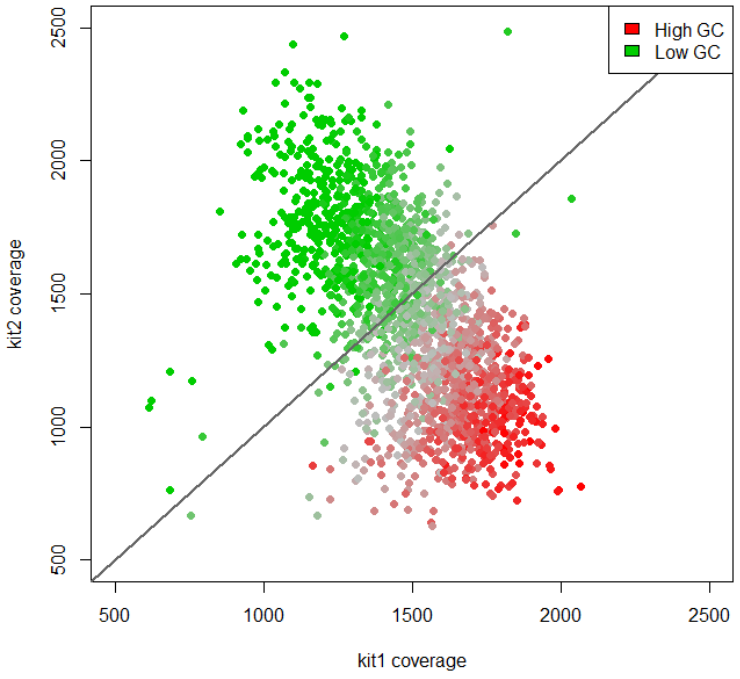
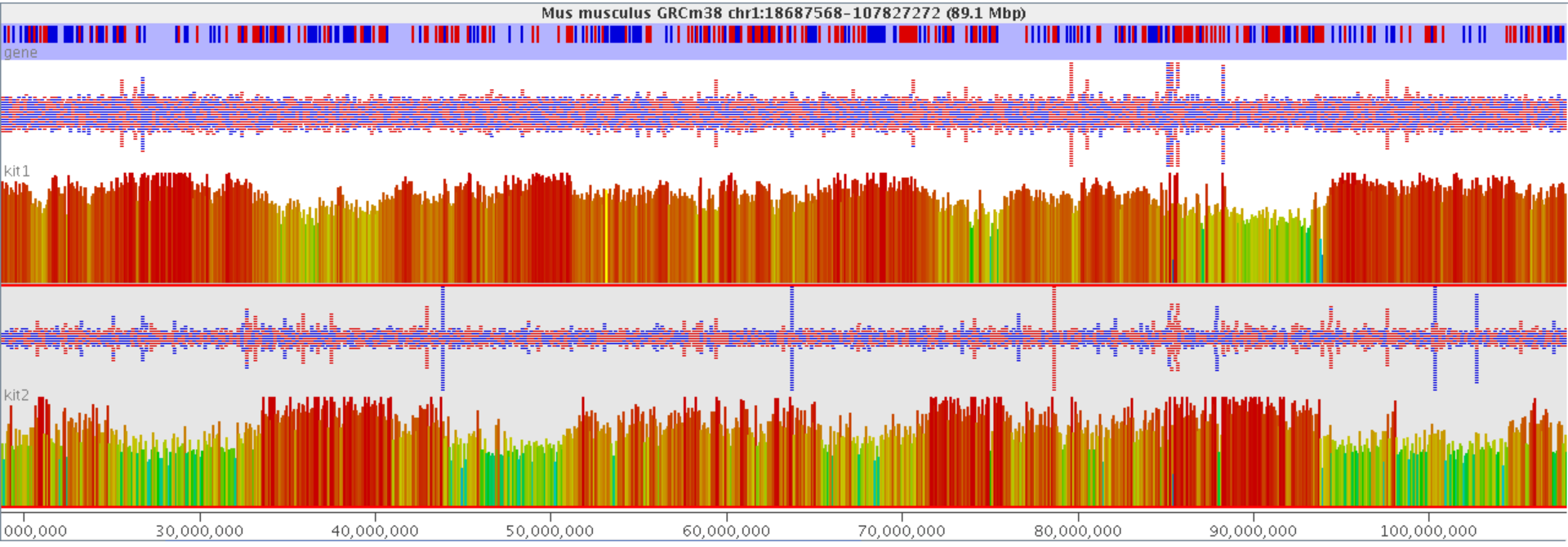
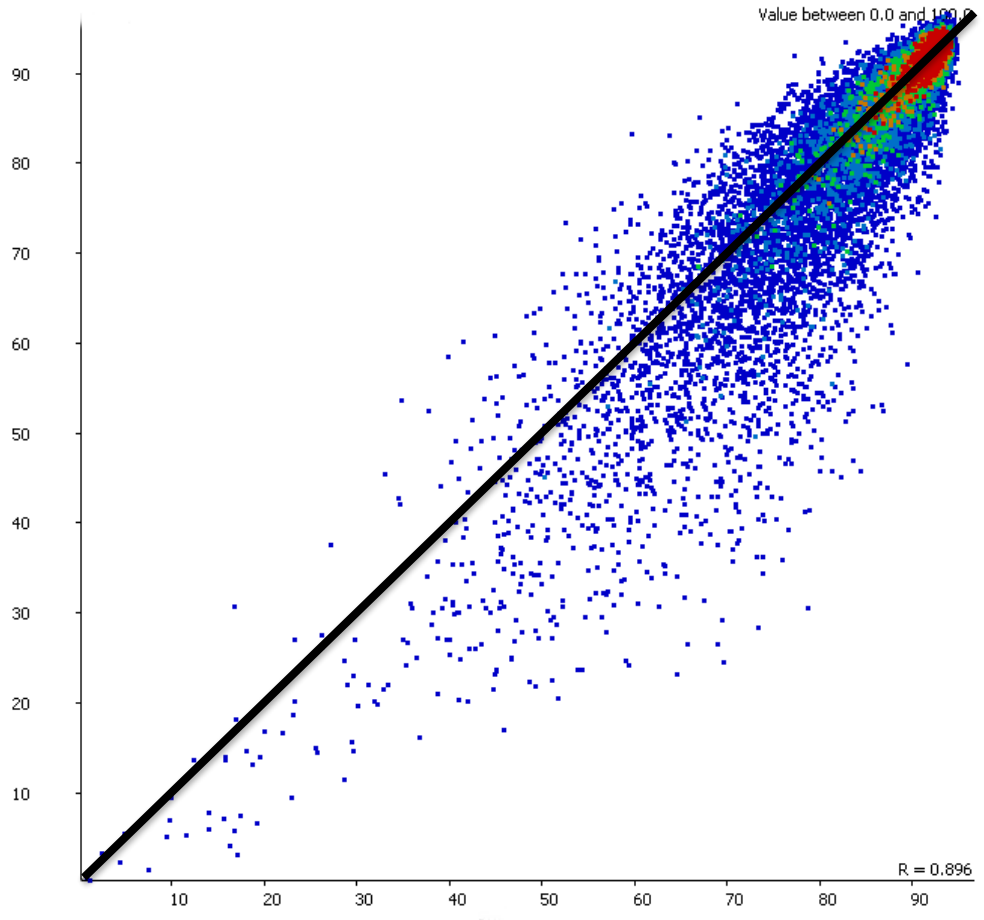
Coverage Bias

GC Content is most likely but others could exist





Coverage bias can lead to Methylation bias



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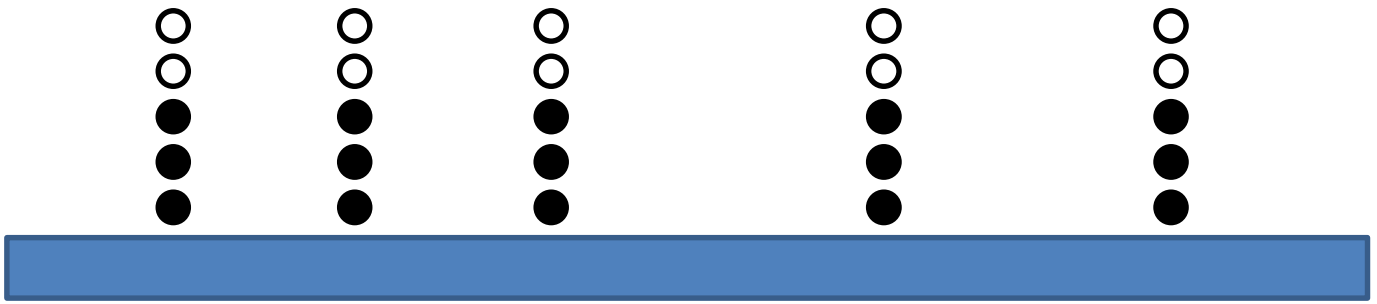
Quantitating Methylation



Total methylated calls = 15

Total unmethylated calls = 10

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

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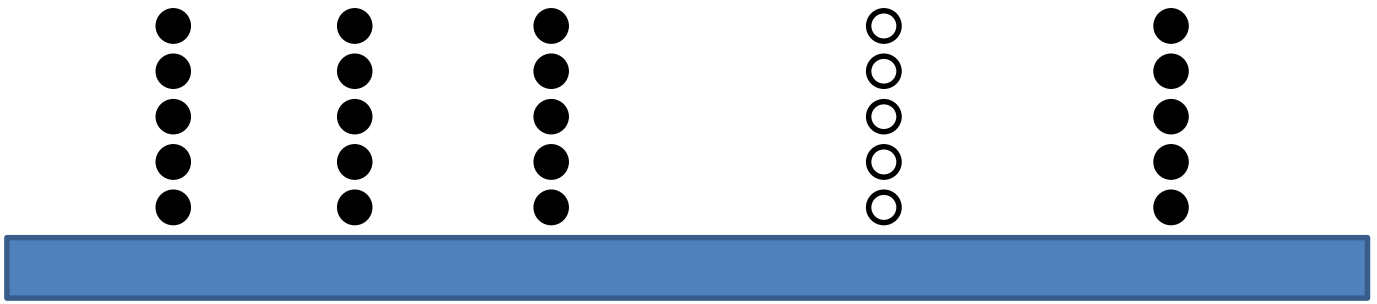
Quantitating Methylation



Total methylated calls = 20
Total unmethylated calls = 5

Methylation level = (20/(20+5))*100 = 80%

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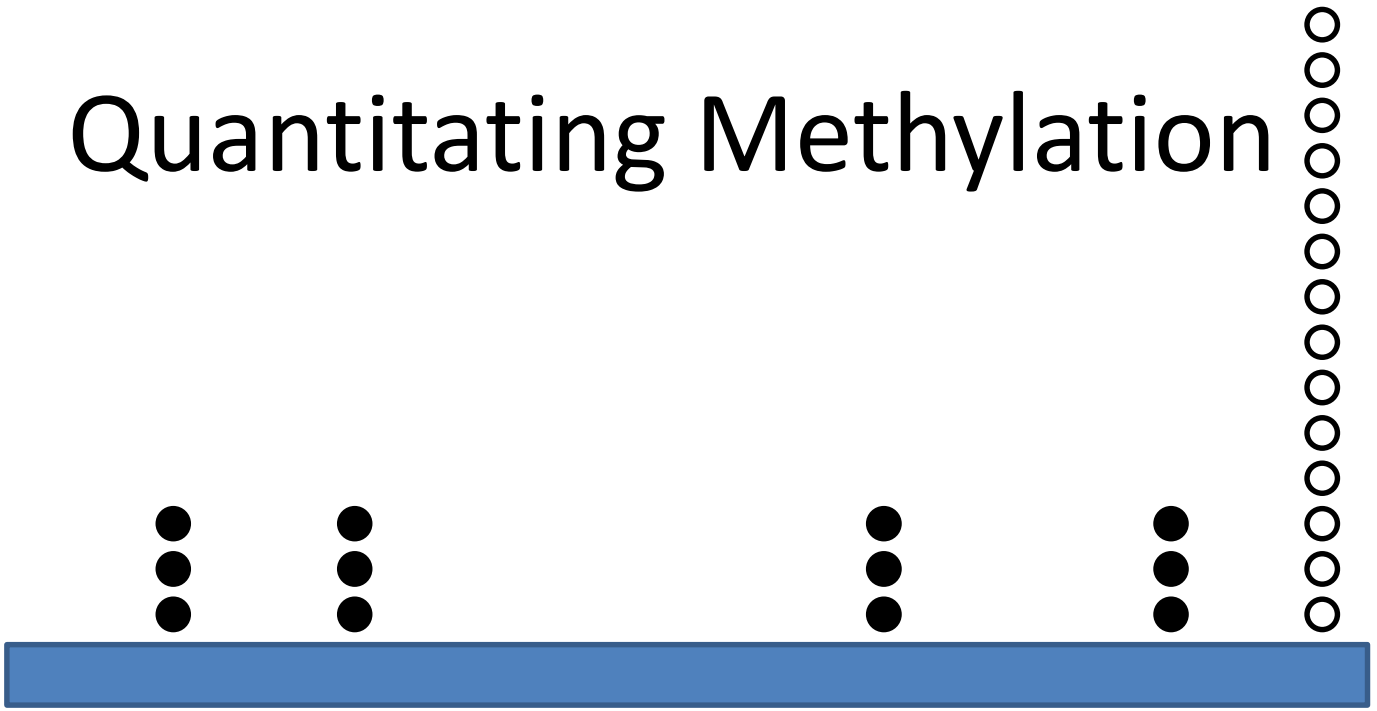
Quantitating Methylation



Total methylated calls = 12

Total unmethylated calls = 14

Methylation level = (12/(12+14))*100 = 46%?

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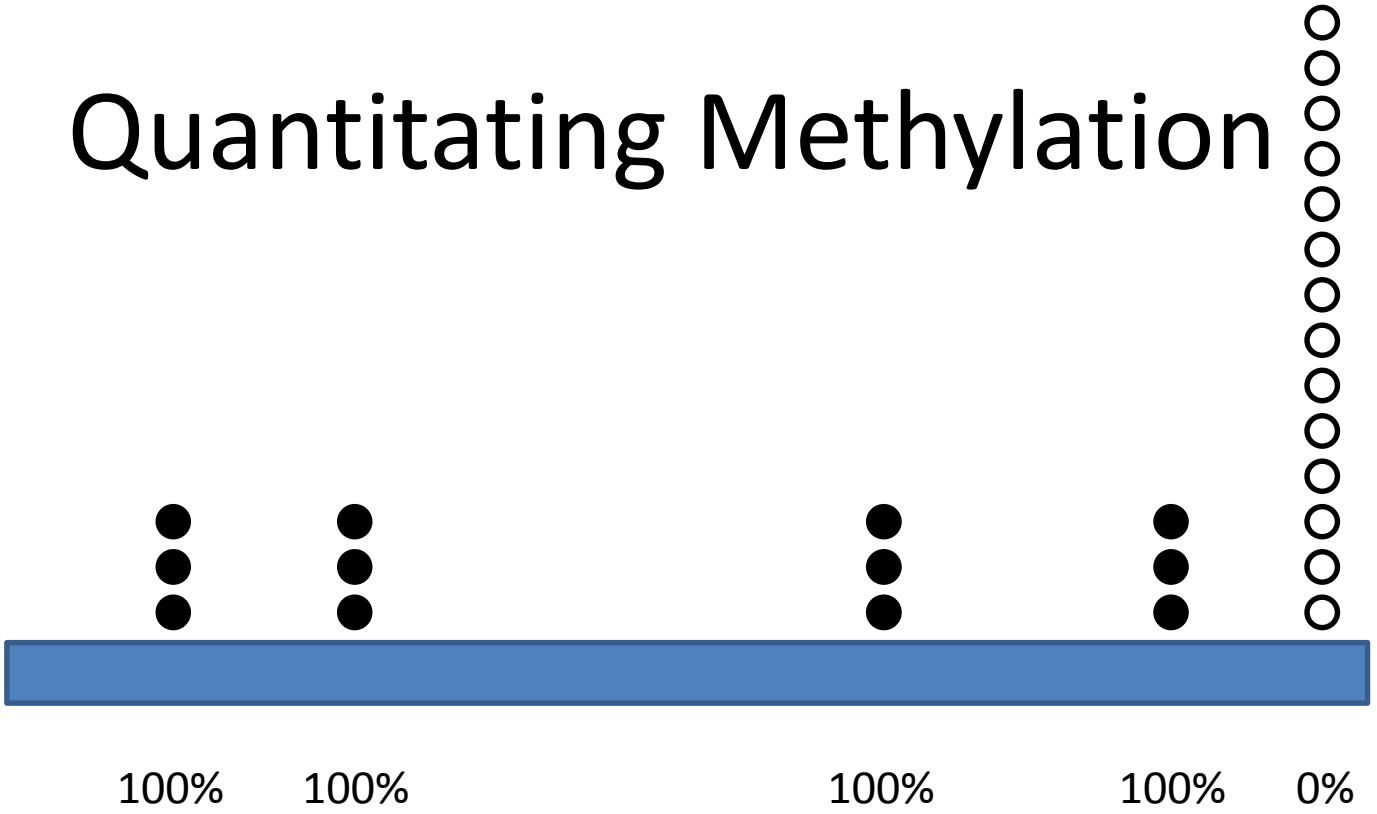
Quantitating Methylation



Methylation level = (100+100+100+100+0)/5 = 80%

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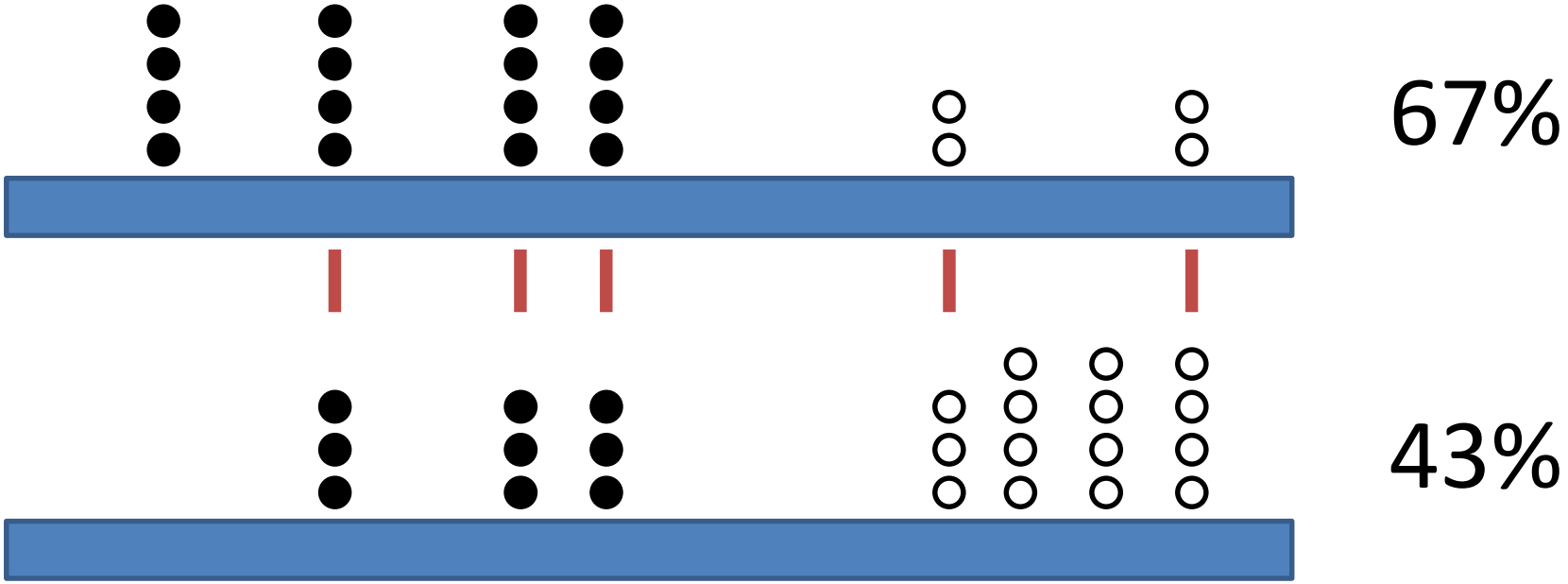
Quantitating Methylation



Common = 60% in both

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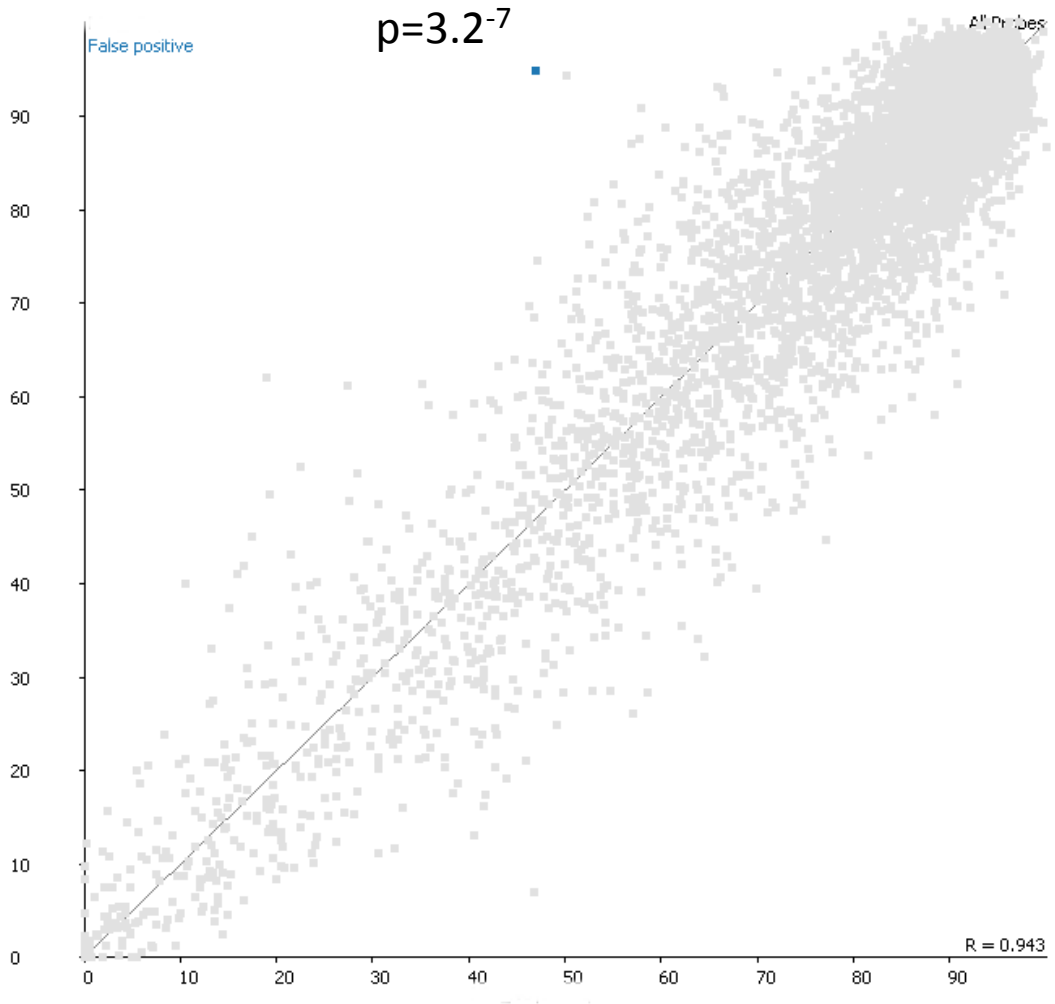
Example



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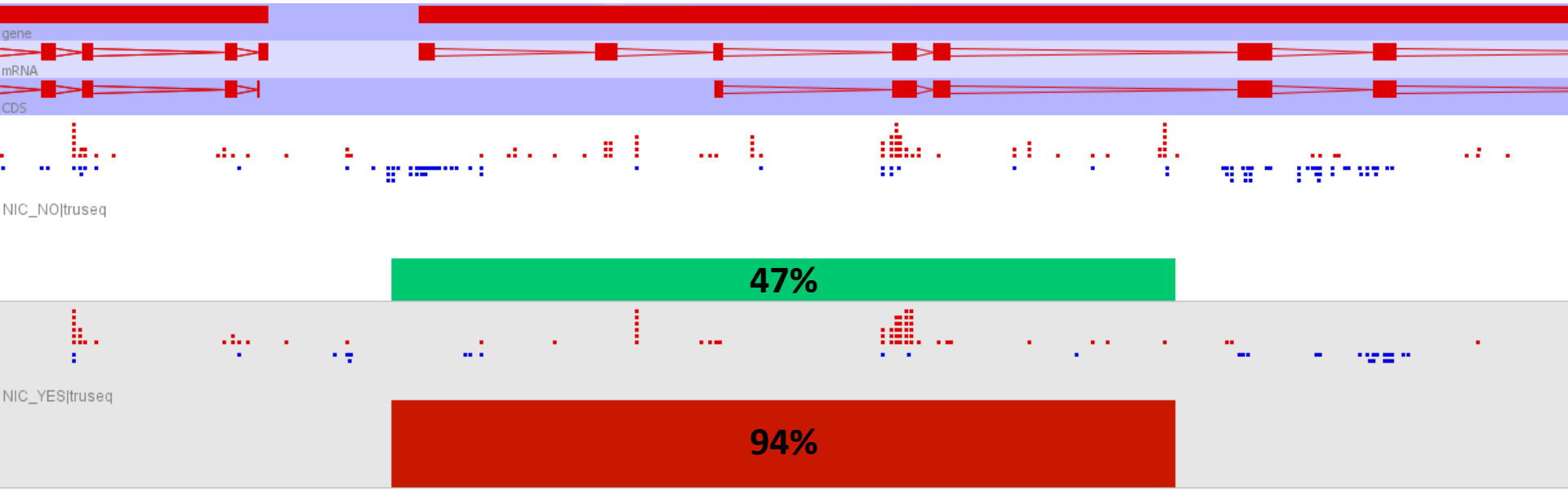
Example



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More Complex Methods

- Factors to consider during quantitation
  - Surrounding methylation levels
    - Assume that methylation doesn't change over very short distances
  - Coverage of individual bases
    - Down-weight very low or high values
  - Density of CpGs
    - Apply the level to all bases within a region

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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

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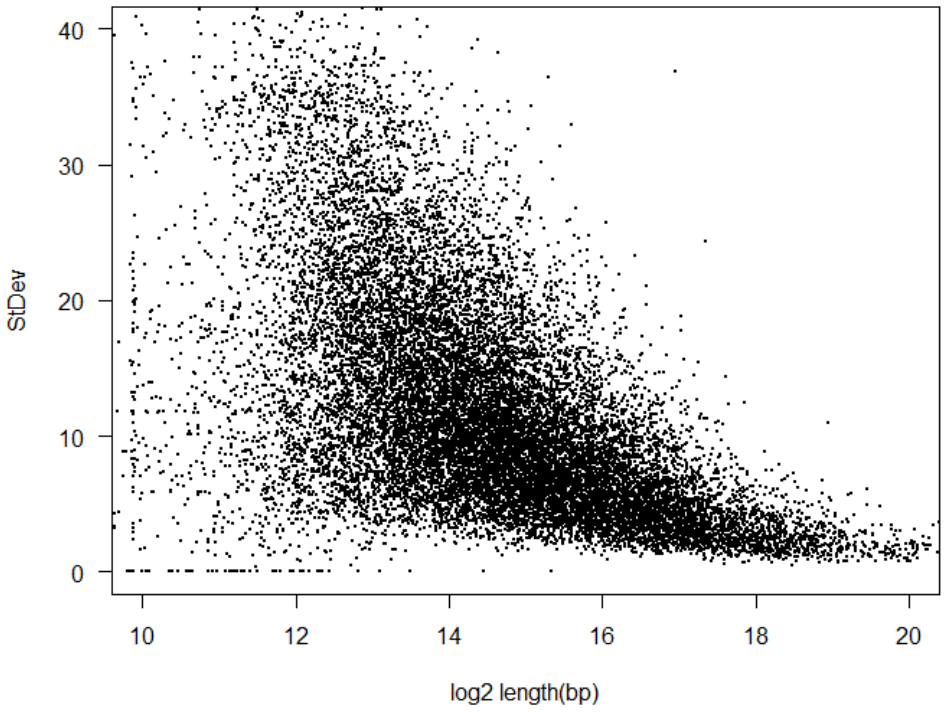
Making Comparable Measures



- 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.

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Unbiased analysis

- What basis do you use for creating the windows?
  - Fixed size?
    - Uneven CpG distribution can be problematic
  - Fixed CpG count?
    - Different resolution

- Need to tailor the window sizes to the data density
- Can relate windows to features later

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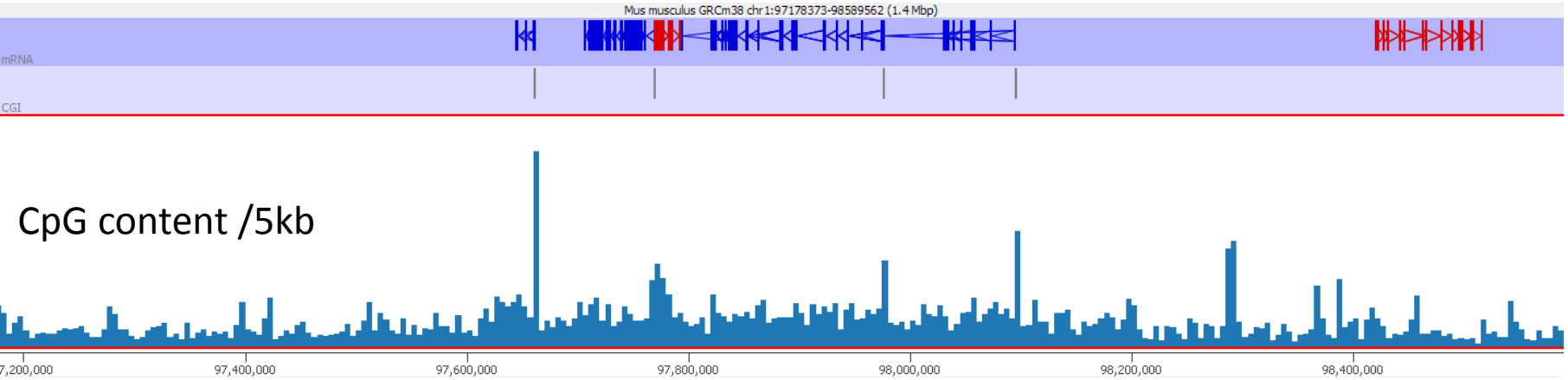
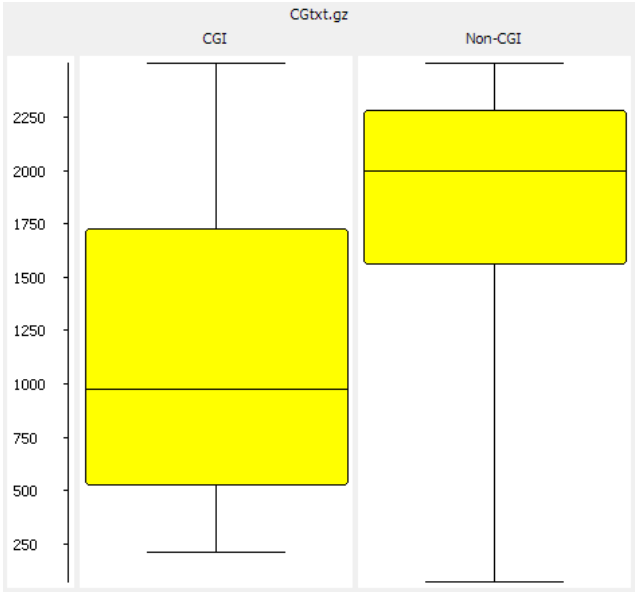
Fixed size vs Fixed CpG





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?

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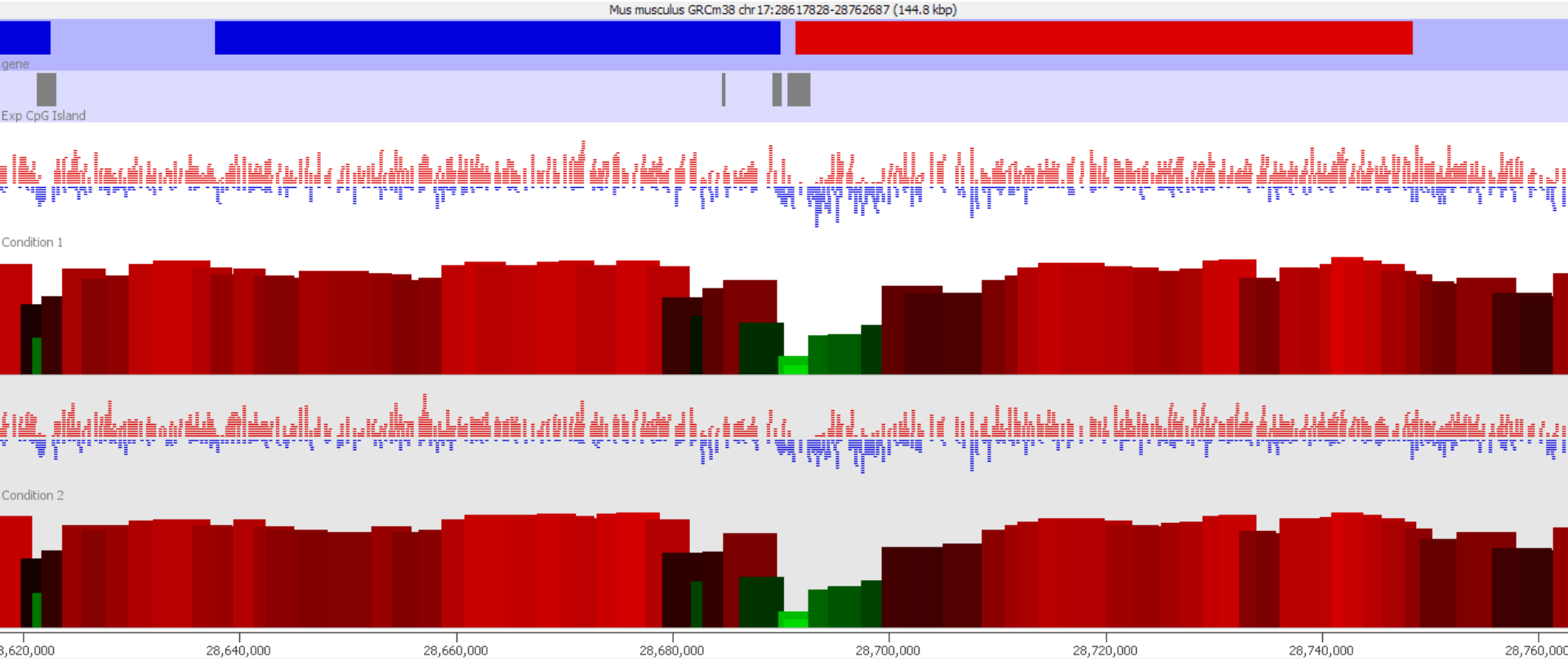
Viewing quantitated methylation



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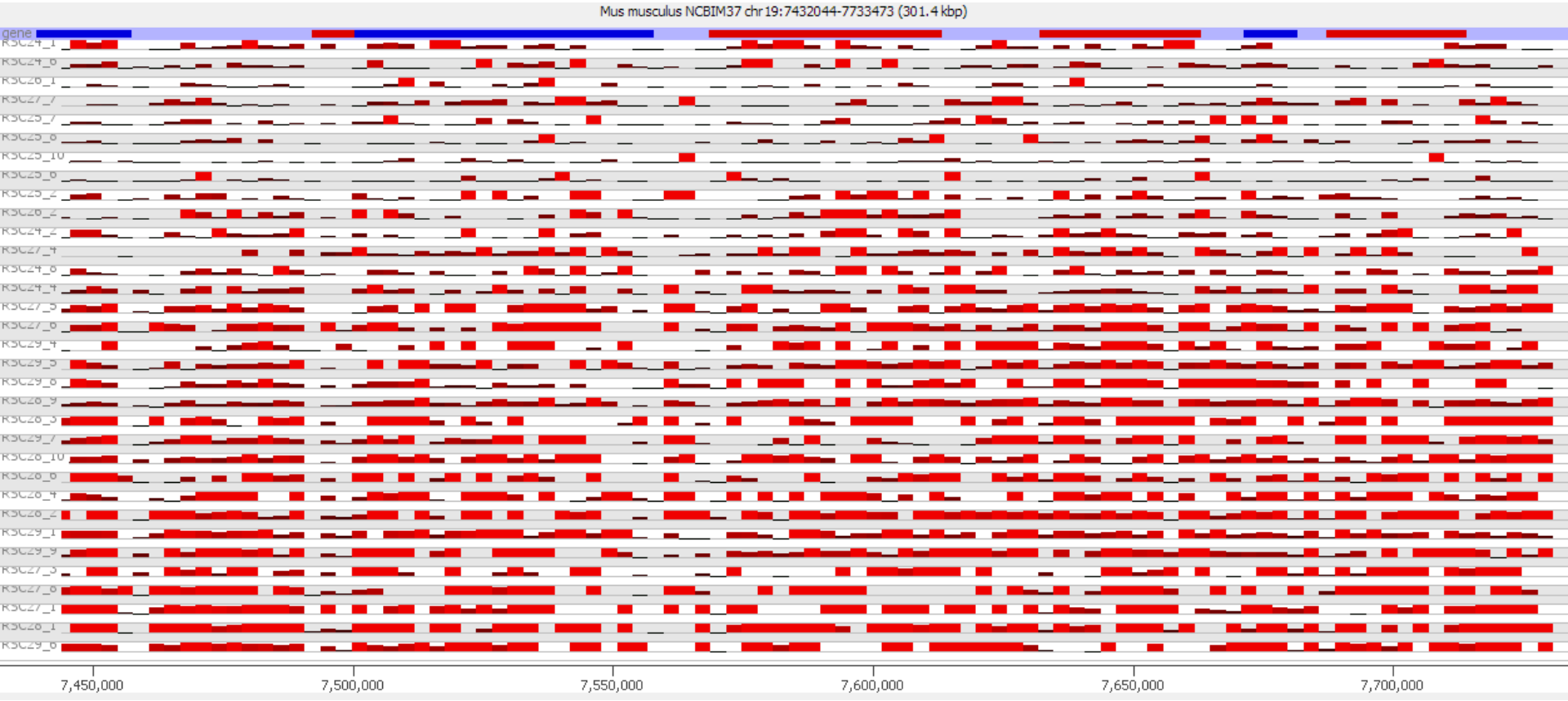
Large sample numbers



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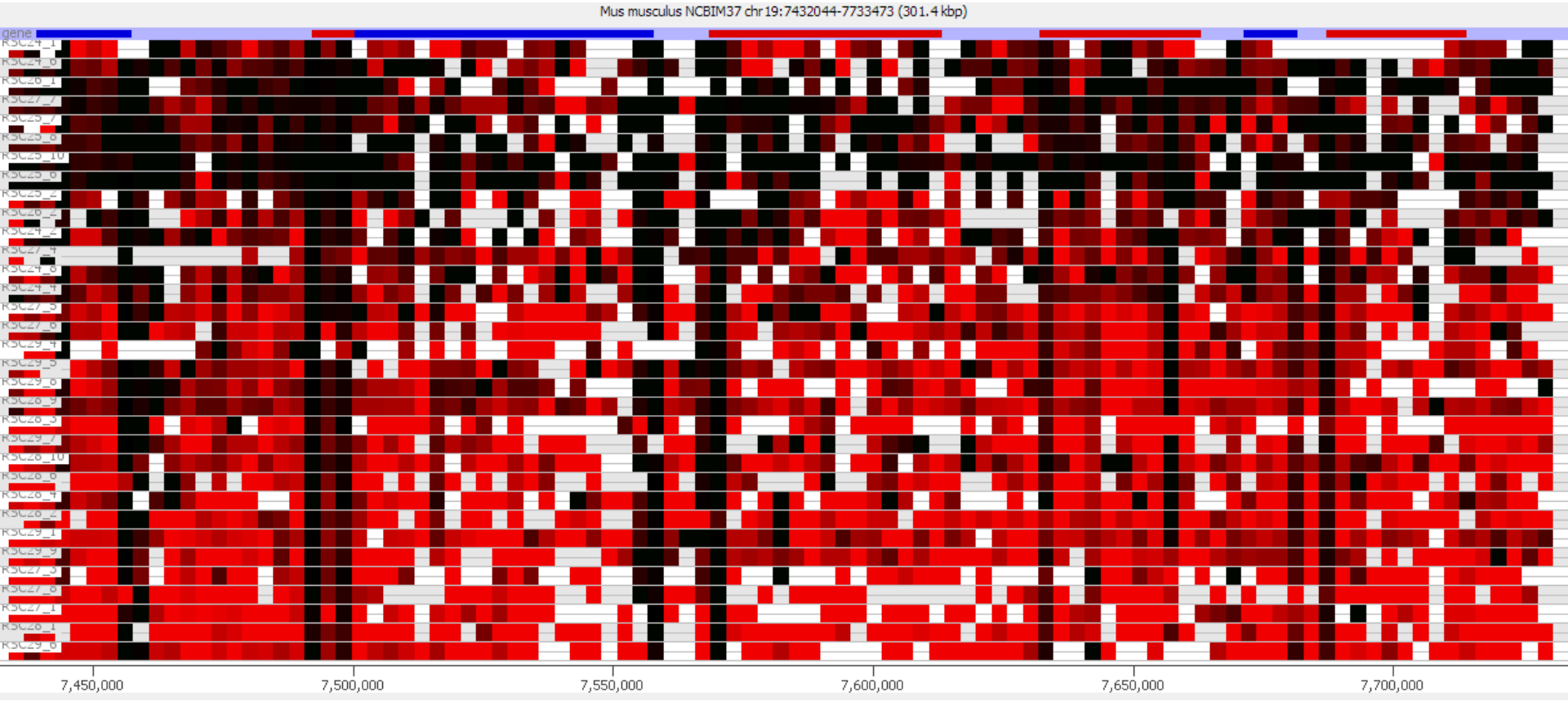
Large sample numbers



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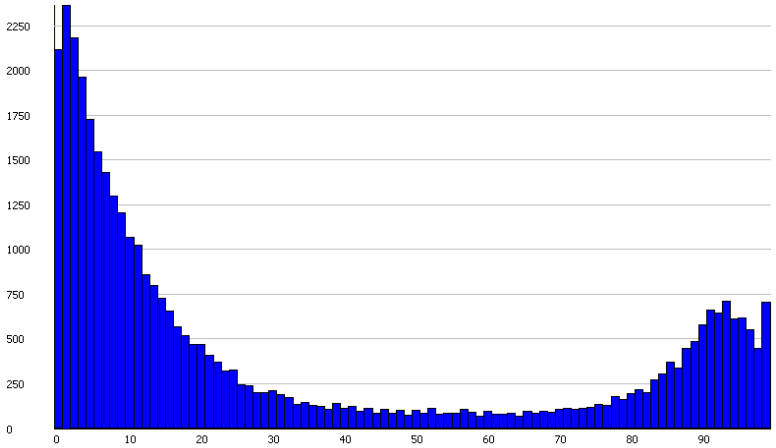
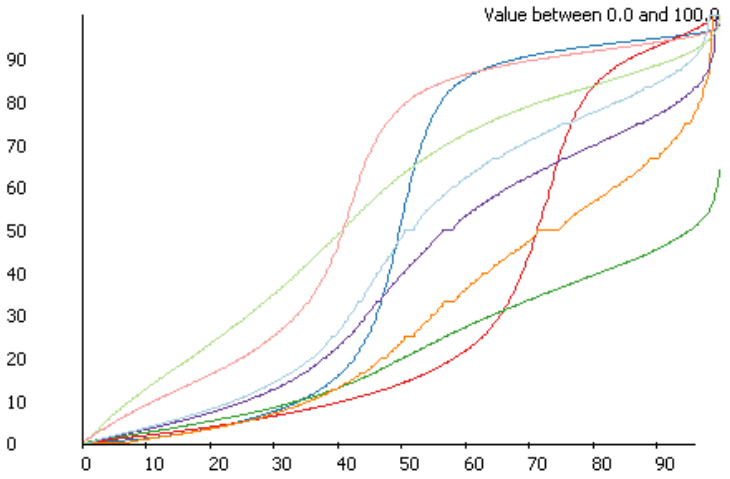
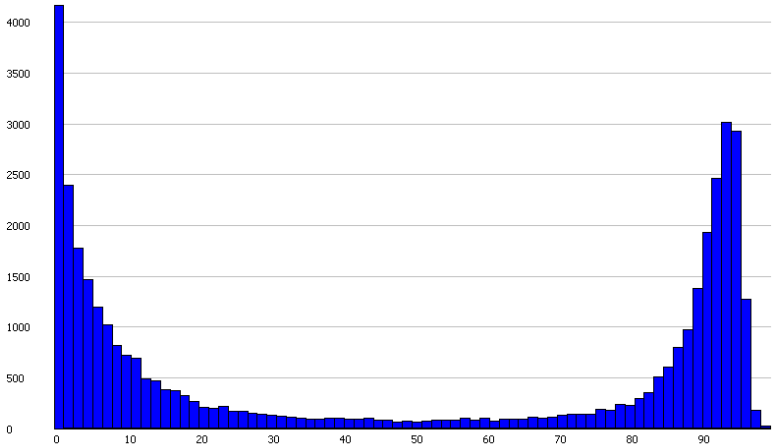
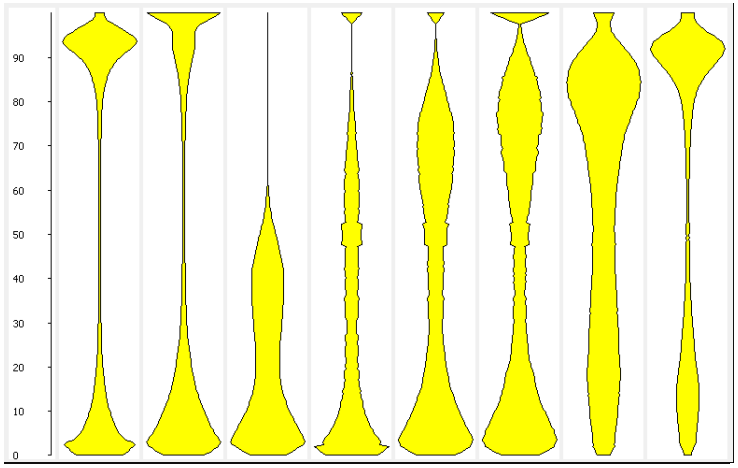
Viewing and Comparing Distributions









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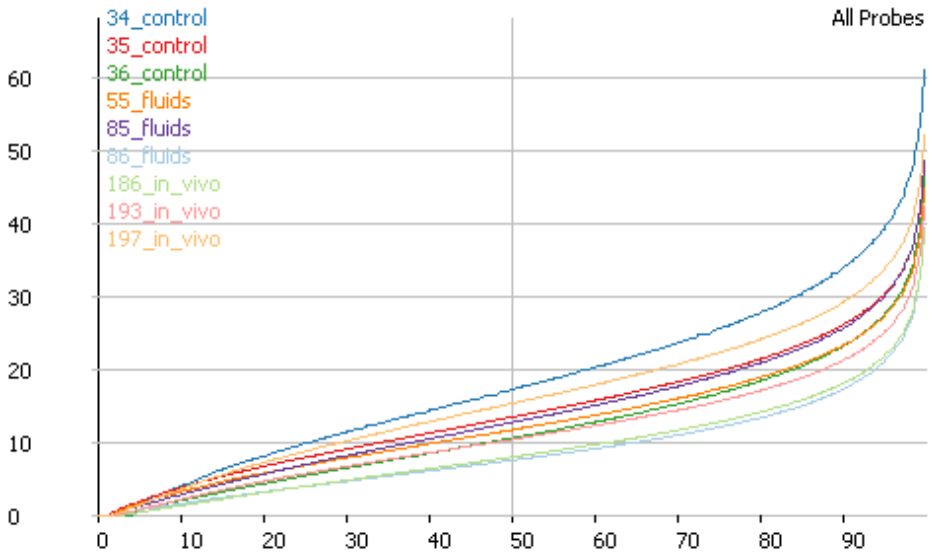
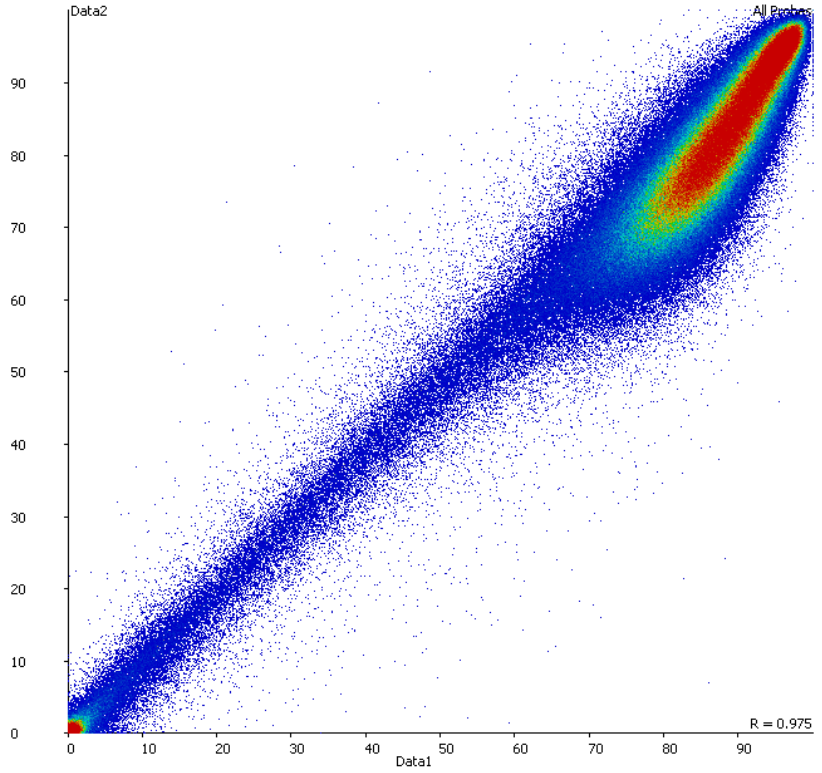
Normalising Methylation Values





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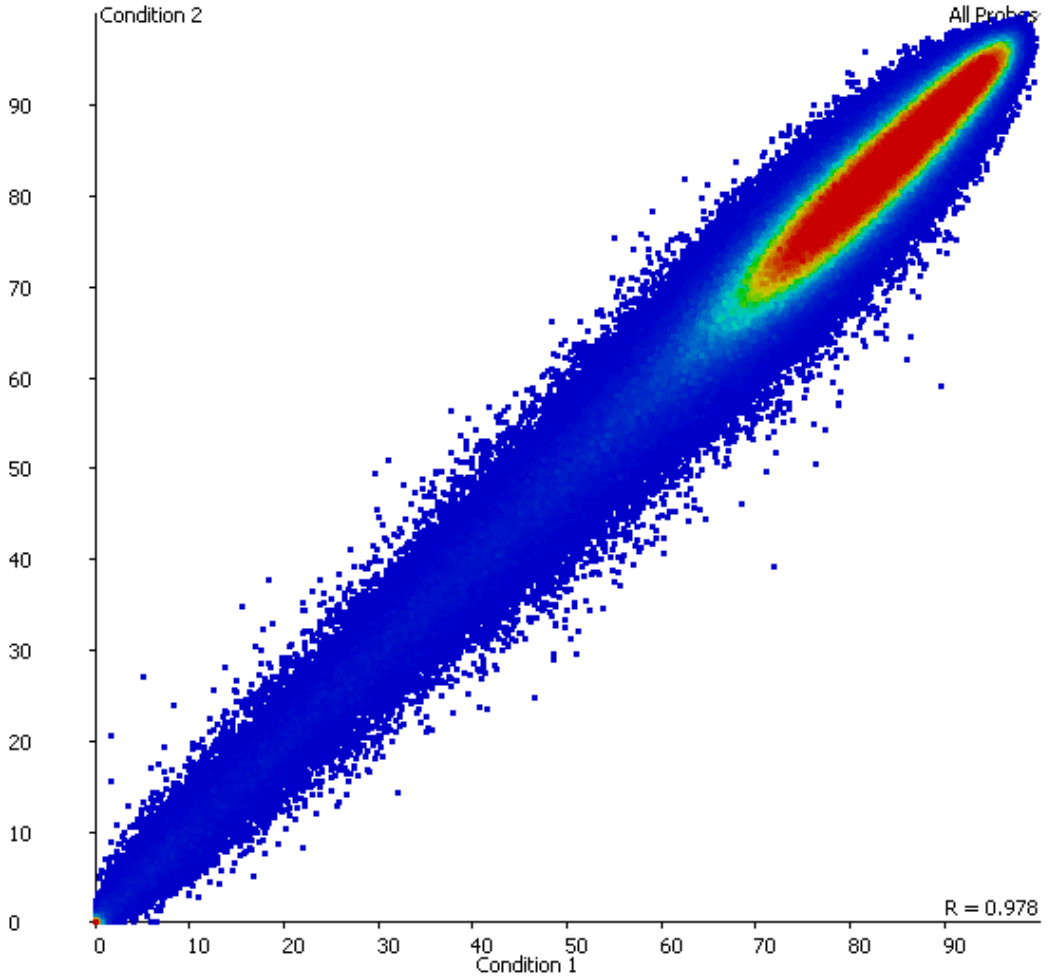
Viewing comparisons



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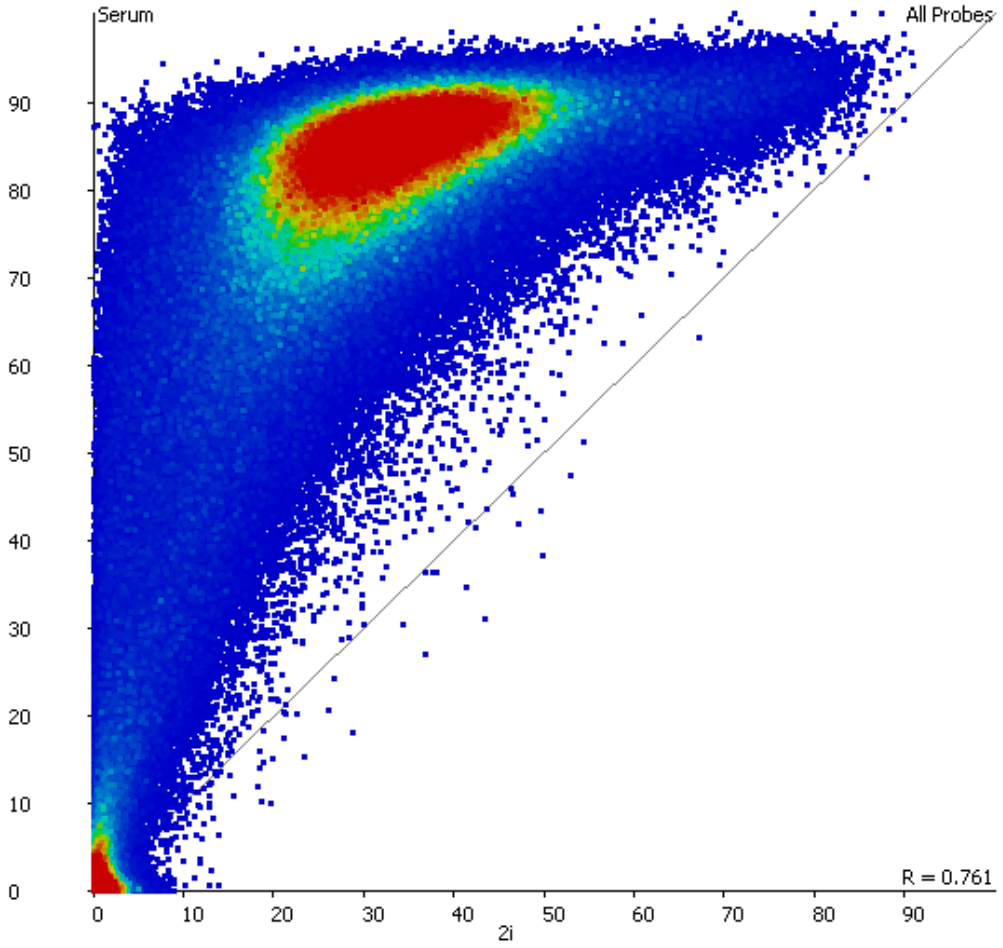
Viewing comparisons



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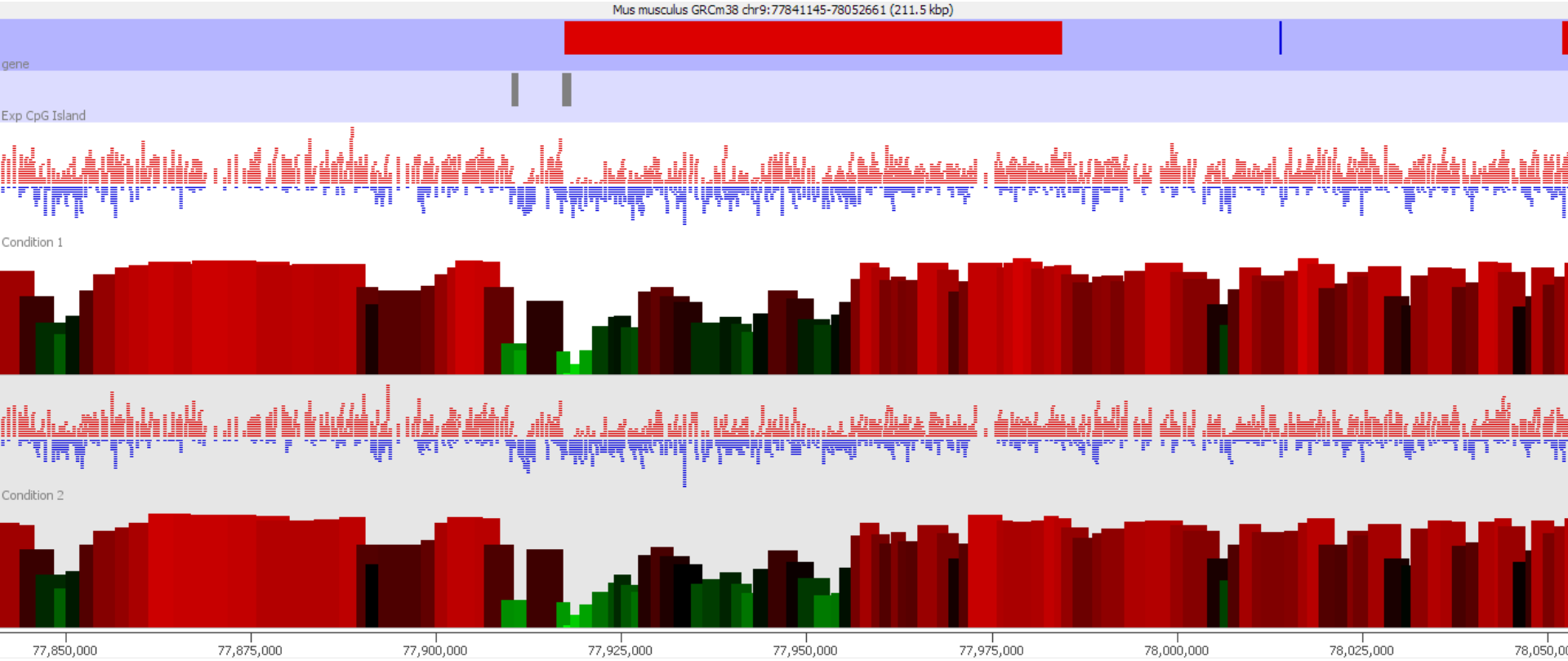
Viewing differences



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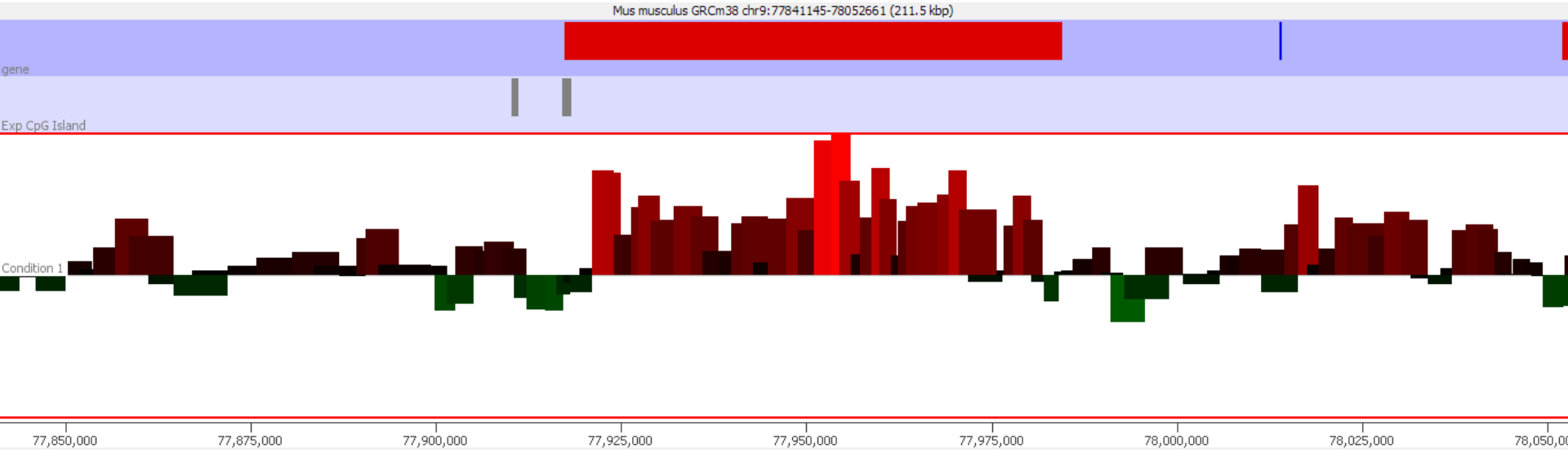
Viewing differences



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Trends

- Effects at individual loci can be subtle
- Want to find more generalised effect
- Collate information across whole genome
- Look for general trend

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Considerations for trend plots

- What features to use
  - Fixed vs relative scale
- How much context
  - Variable scales
- How to calculate base measures
  - What window size
  - Aligned vs unaligned windows
- Missing values
- Scale

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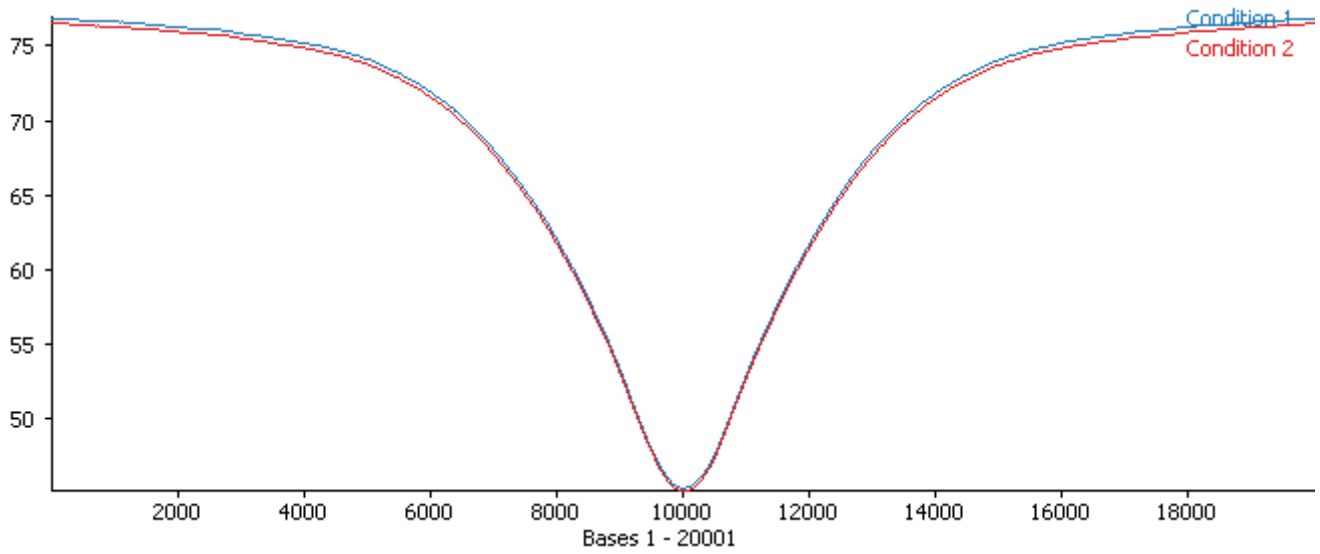
Simple Example



Methylation profile centred on CpG islands +/- 10kb

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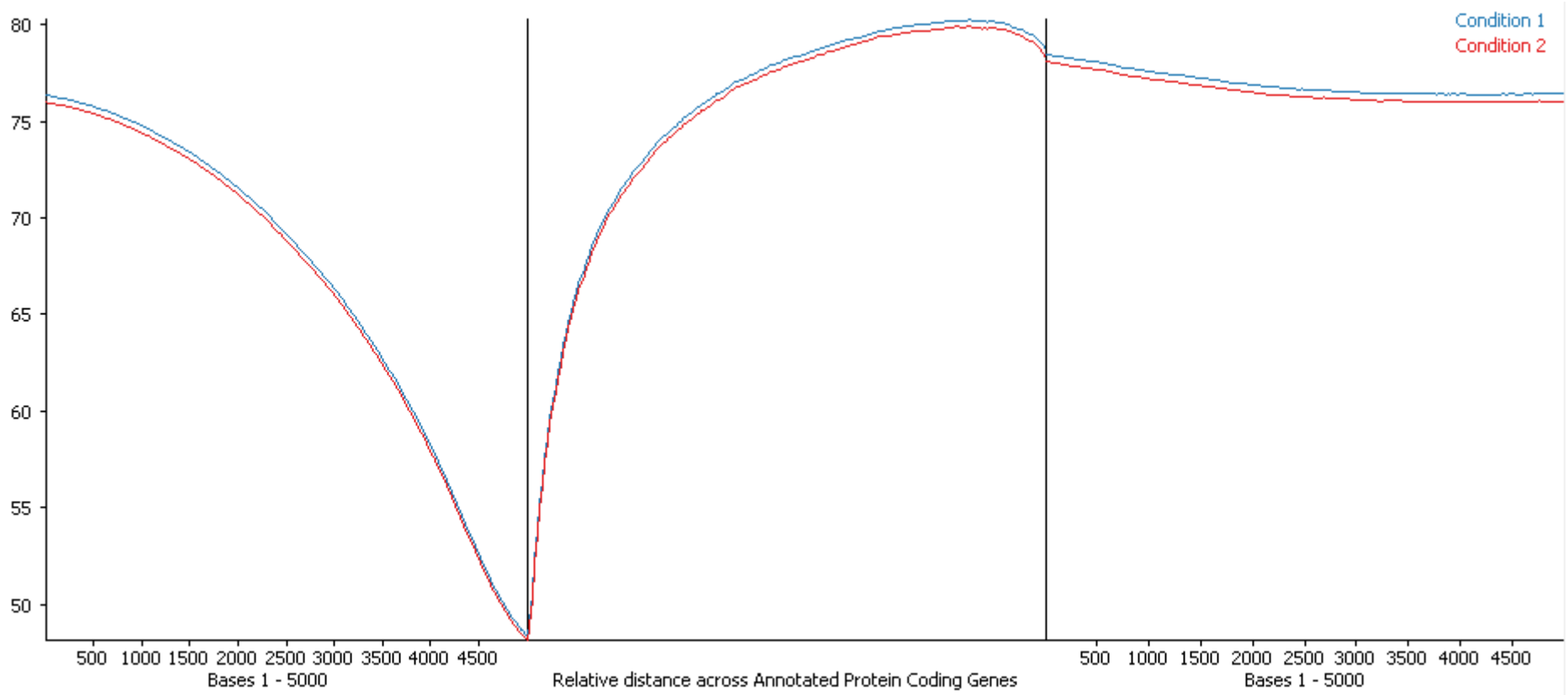
More complex example



Methylation profile over genes +/- 5kb

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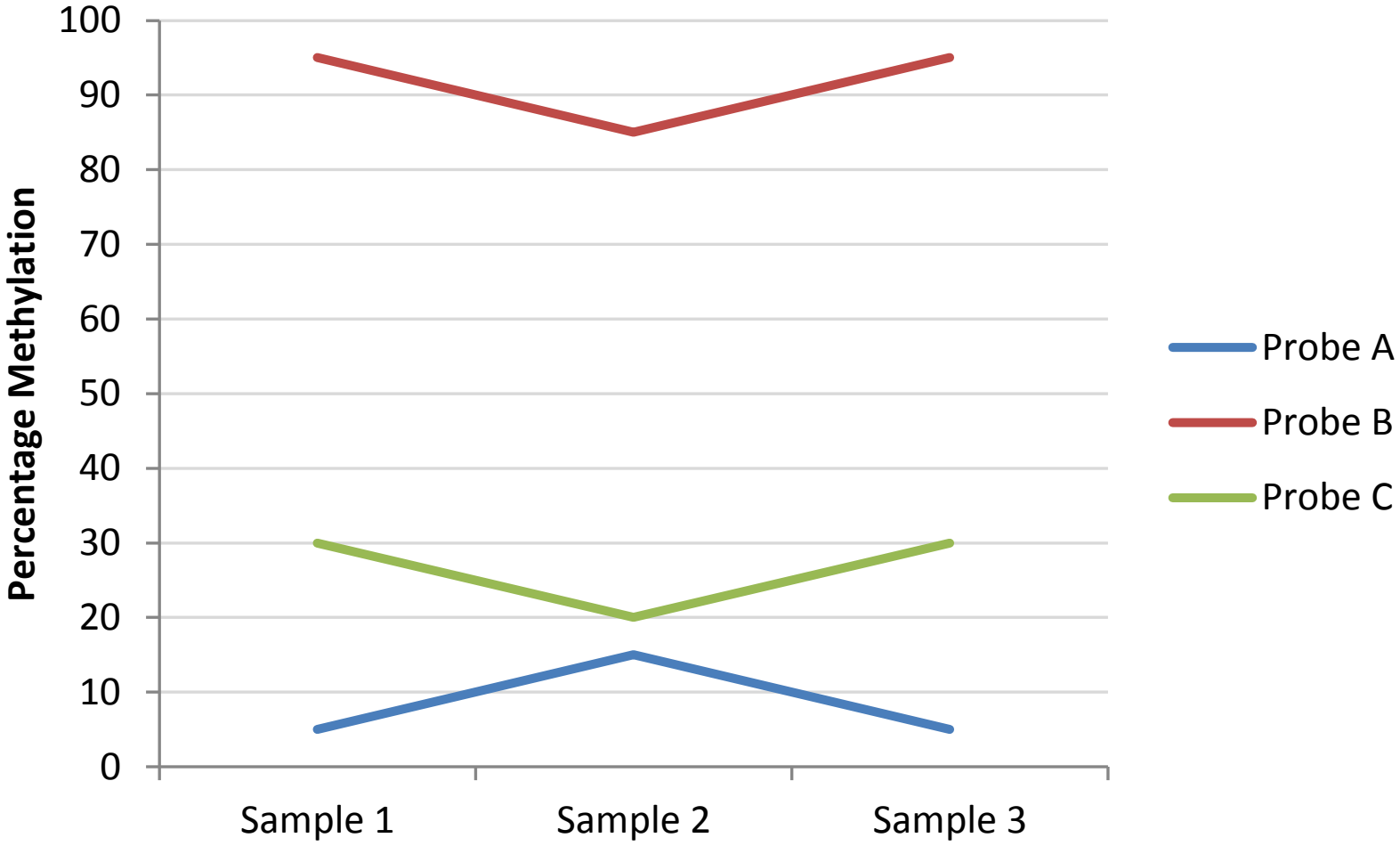
Clustering



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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

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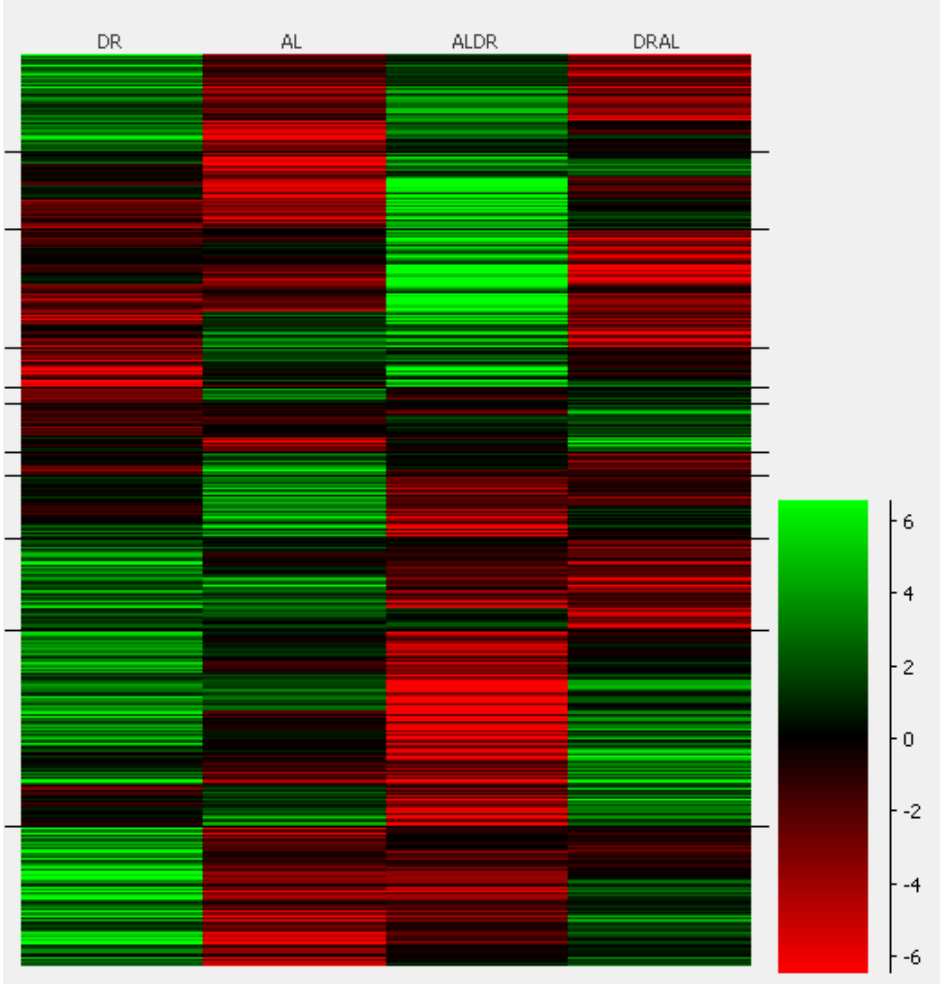
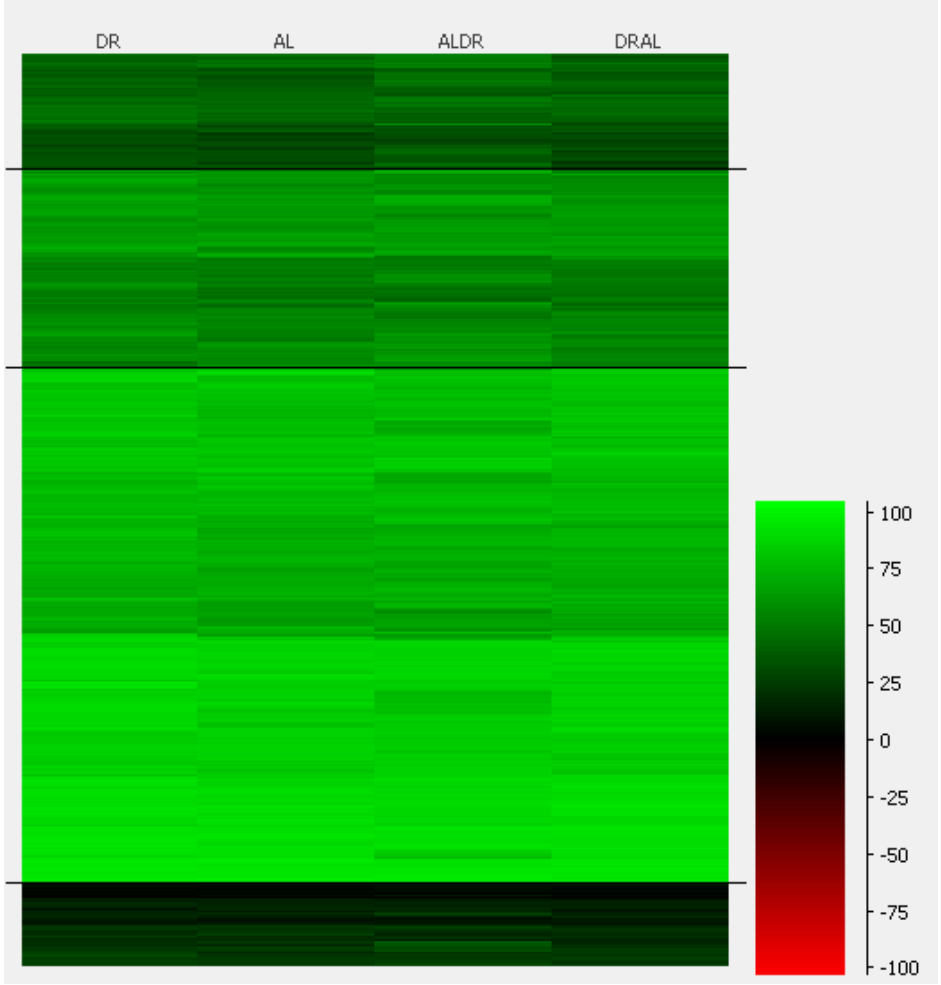
Clustering





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