How Sequencing Experiments Fail

v1.0

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Classes of Failure

Something went wrong with a machine

Samples aren't what they're supposed to be

Problems during sequencing library preparation

Unexpected material in your libraries

Samples didn't behave the way you expected

Drawing the wrong conclusion from the data

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Technical

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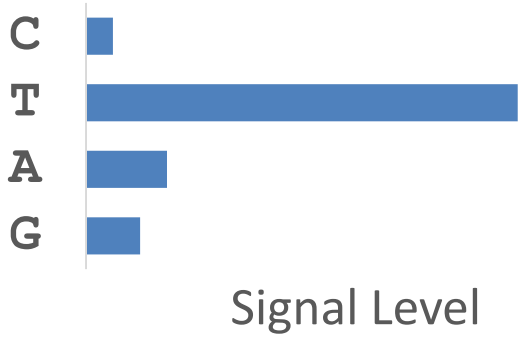
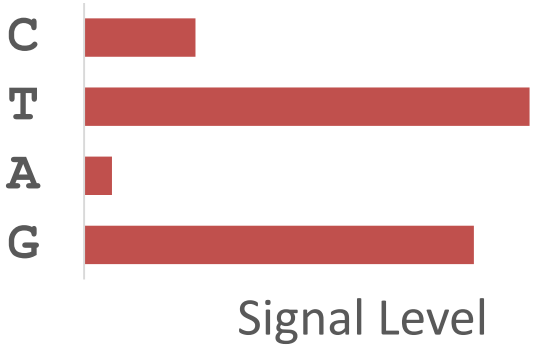
Technical

Technical Failures



Call = T

Confidence = High



Call = T

Confidence = Low

Call = T

Confidence = Low

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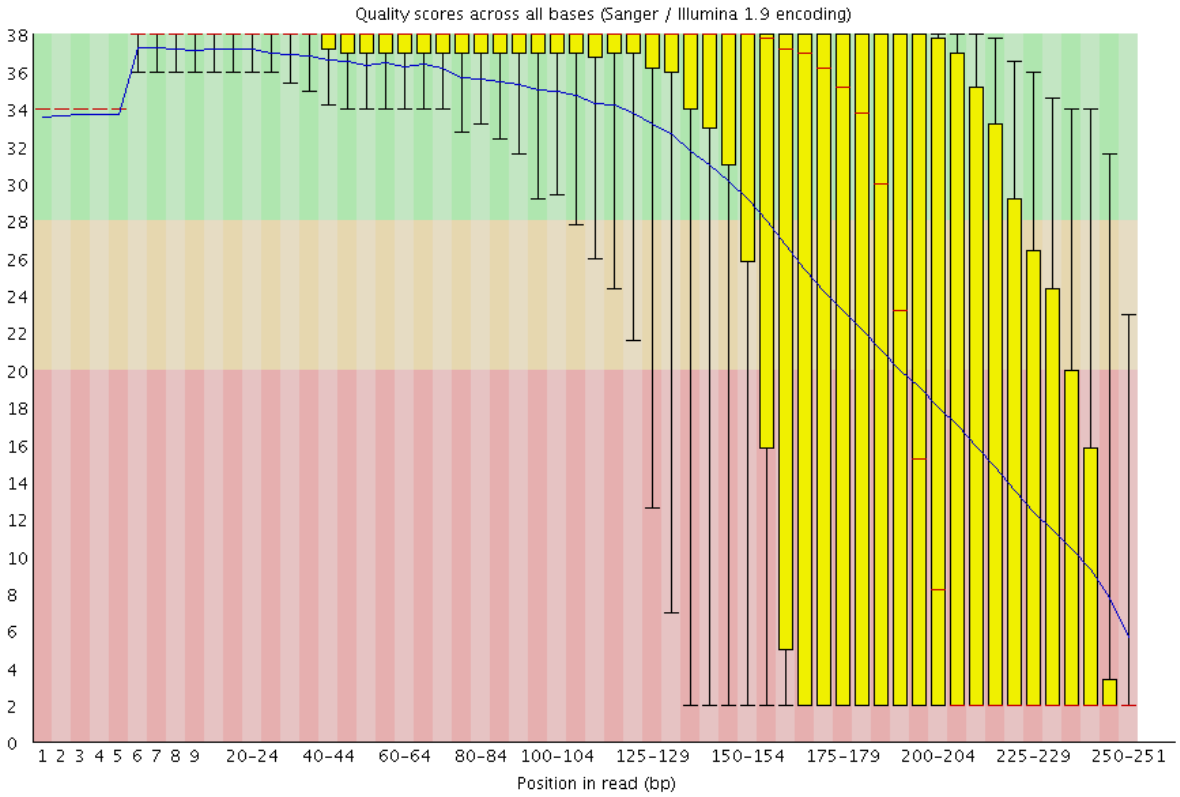
Technical

Phred Scores

$$\text{Phred} = -10 \log_{10} p$$

p = Probability call is incorrect

10% error	Phred10
1% error	Phred20
0.1% error	Phred30



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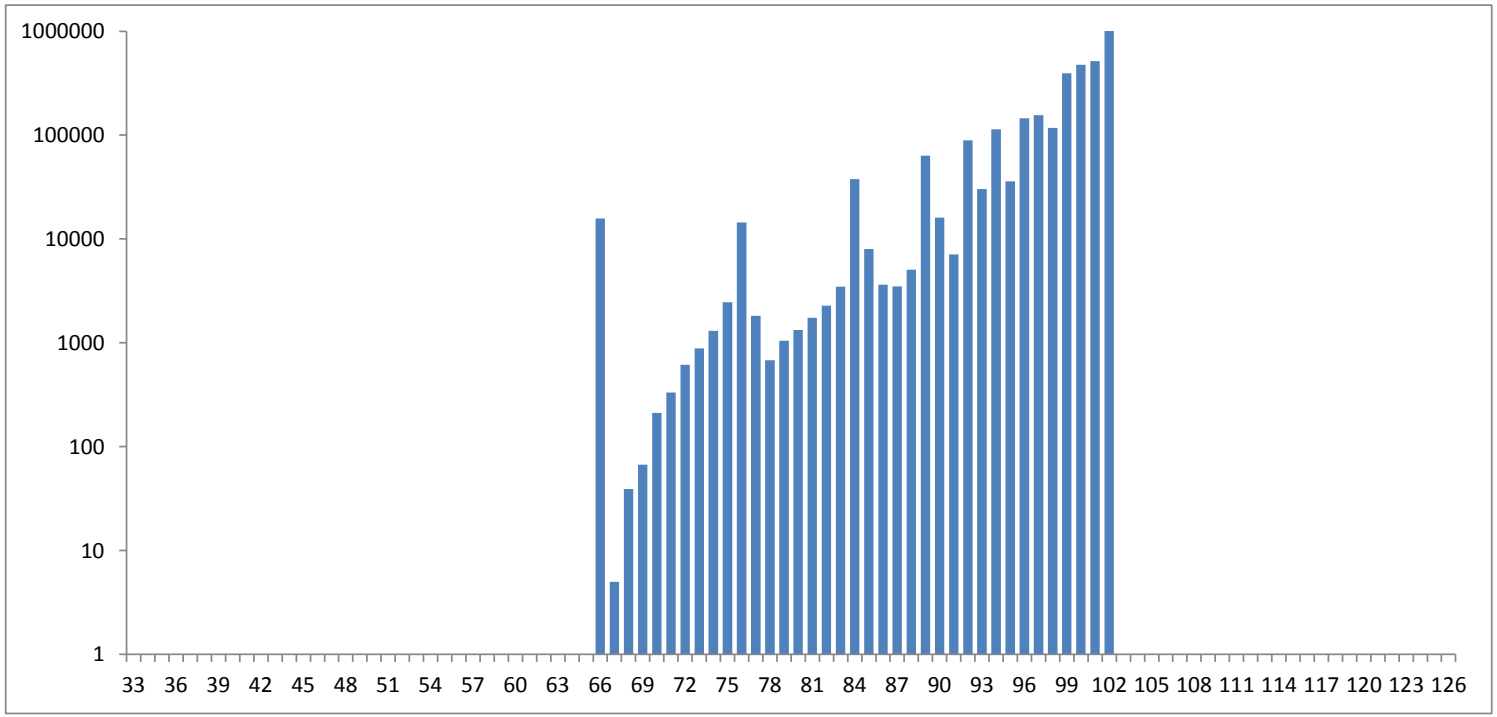
Incorrect Encoding

Phred64

```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefgh
```

Phred33



Phred64 (Illumina)

Phred33 (Sanger)

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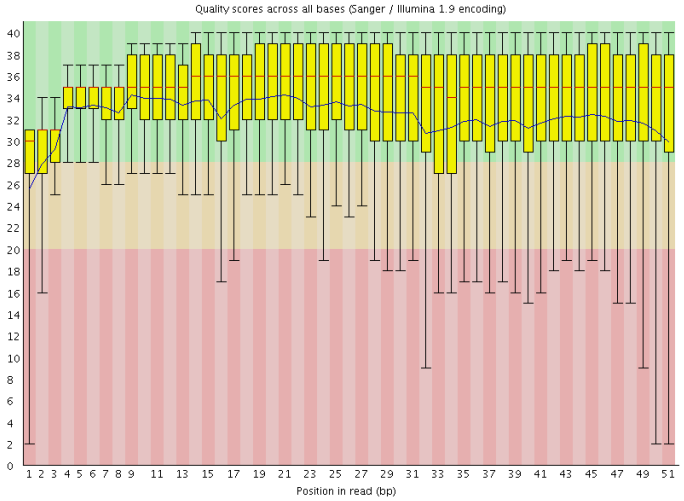
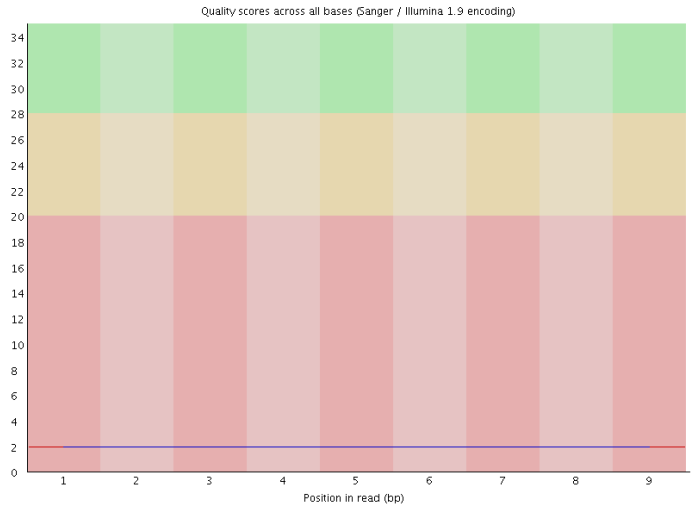
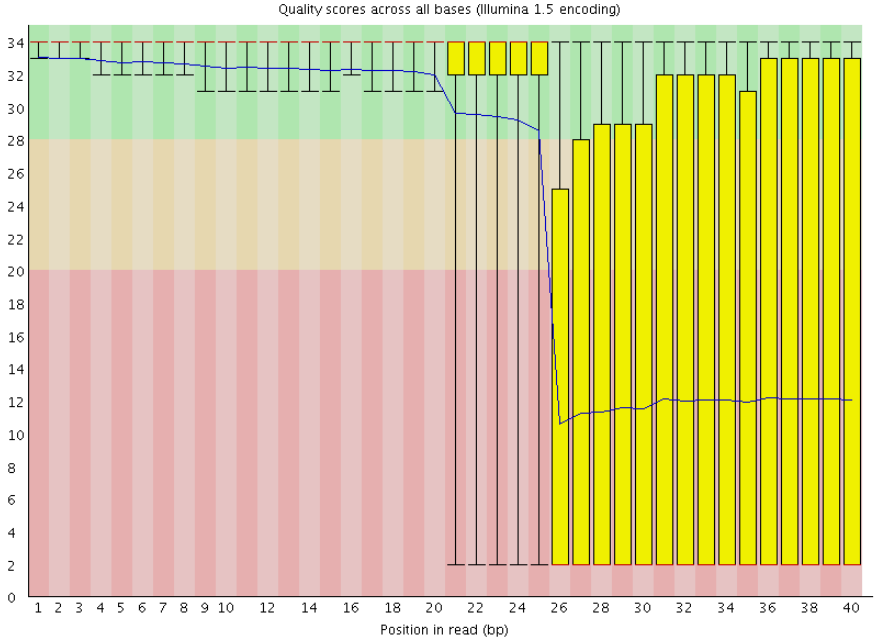
Phred Scores







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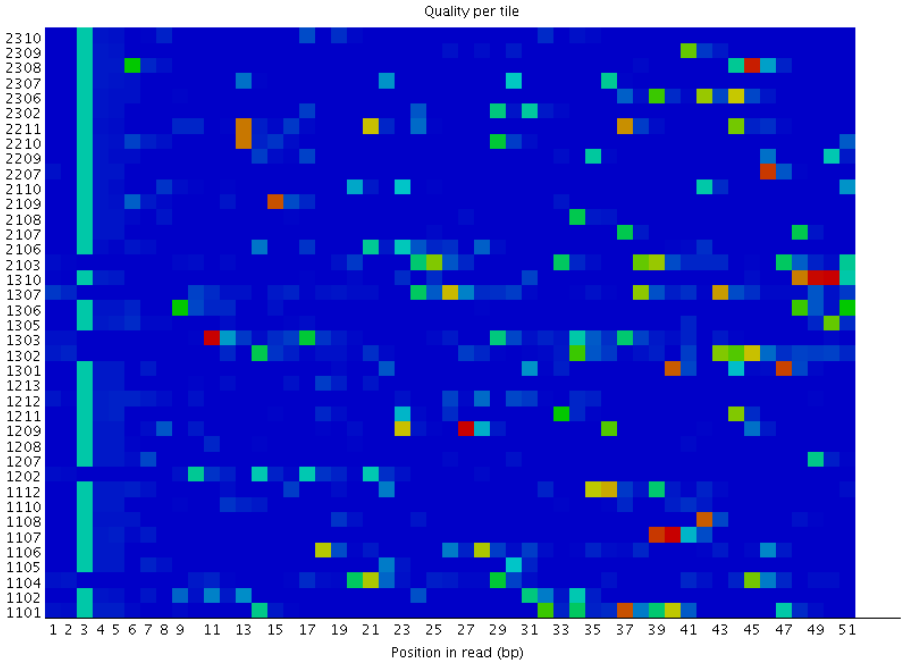
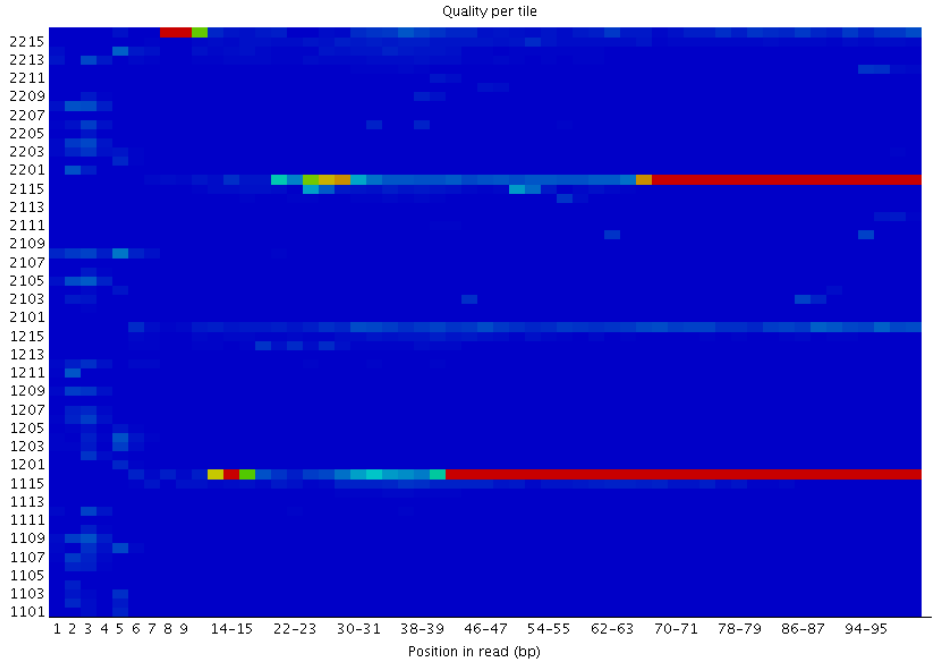
Positional Phred Scores





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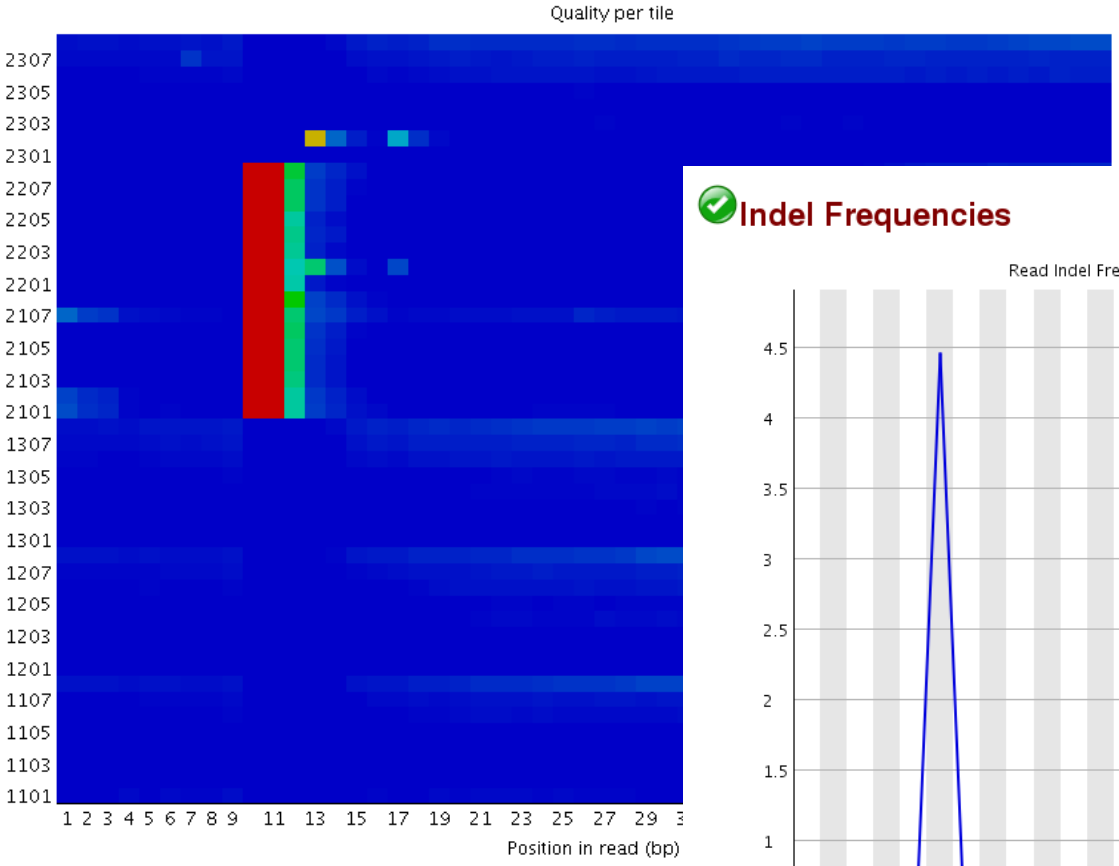
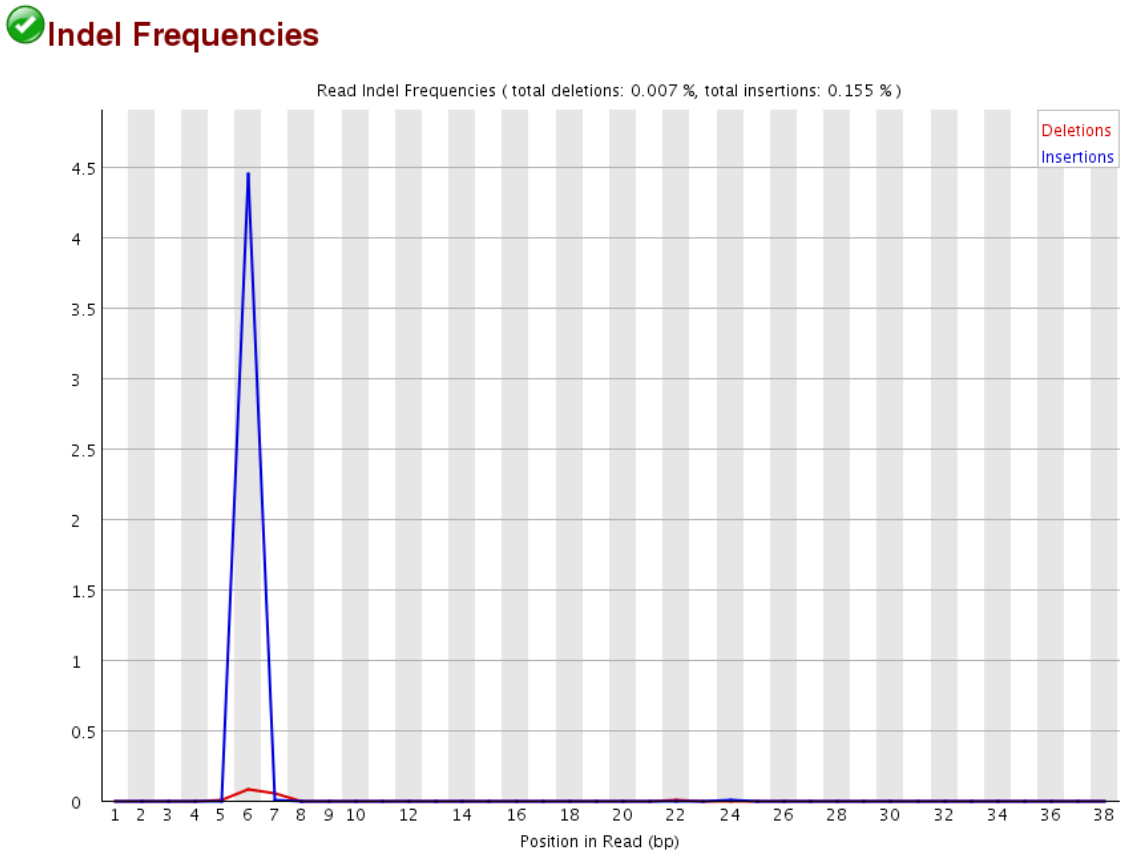
Positional Phred Scores



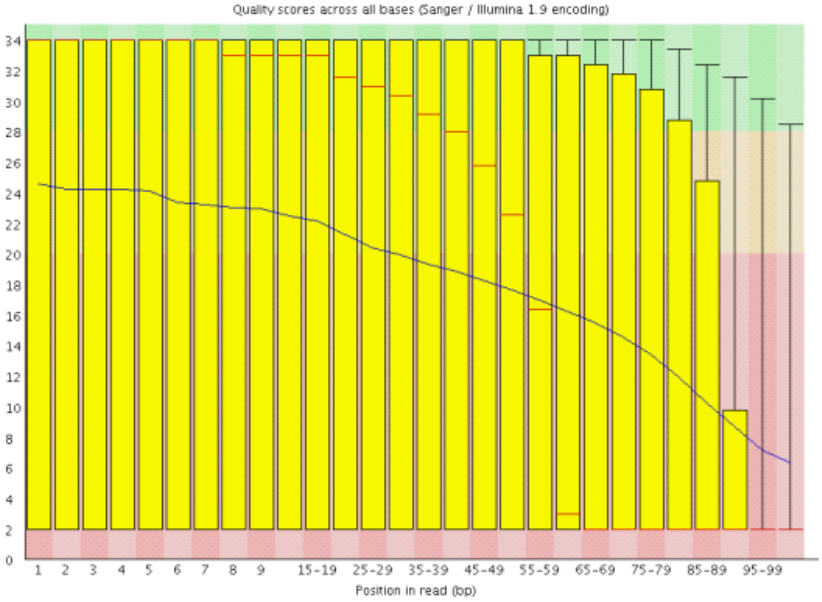


Technical

Biased Phred Scores

HindIII sites

GGATCCGTATGCGATGCTAGCGT
GGATCATATATATGCTAGCGTAT
GGATCTATATTGCGCGATACTGG
GGATCCCGTAGCTGCGATGCTGA
GGATCAAGGATAGCGCGTCTAGA
GGATCTATATAGTTGCCGTATCG
GGATCGGAGCGGGATCGGATGCG



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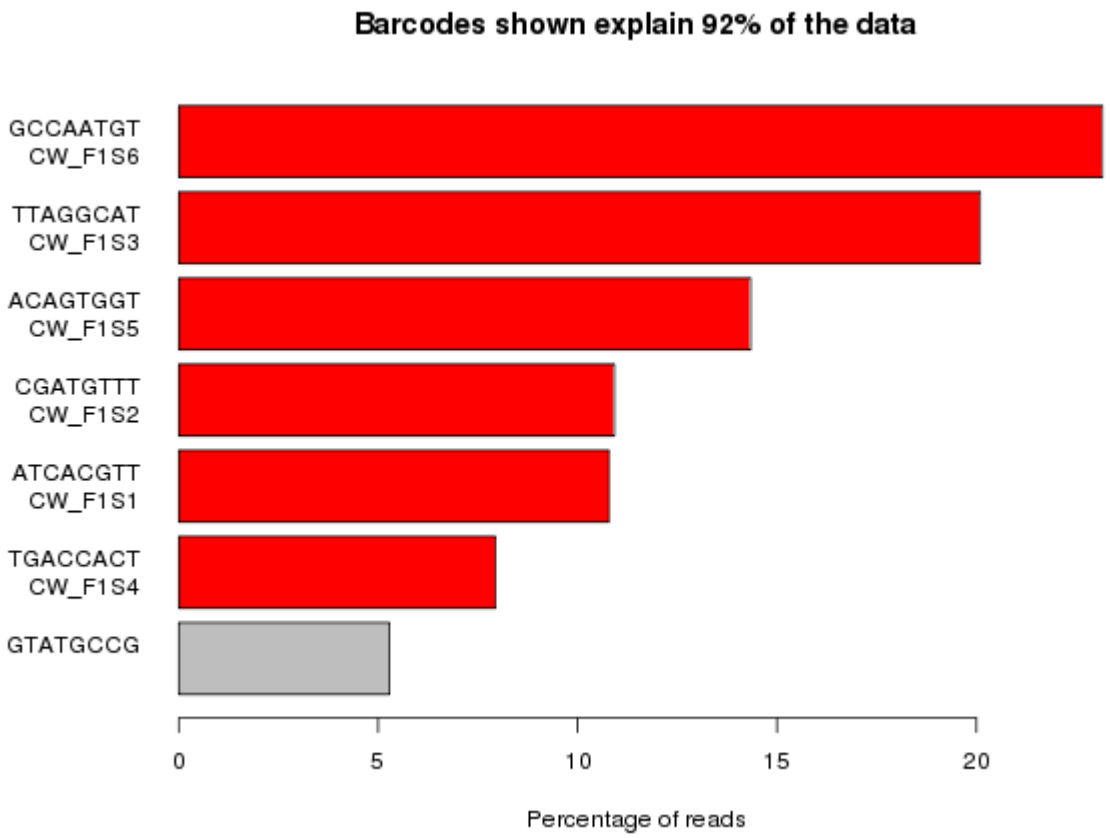
Tracking

Tracking

Tracking - Barcodes



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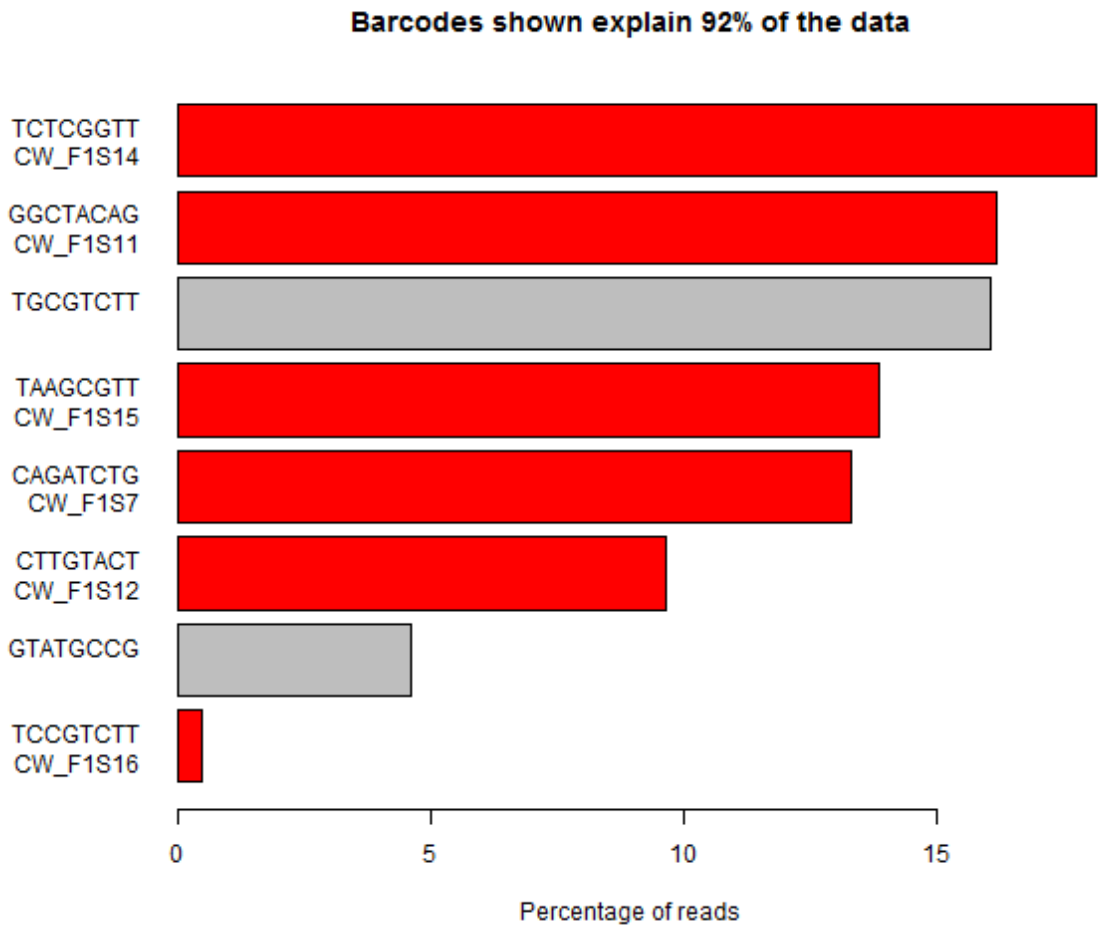
Tracking

Tracking - Barcodes



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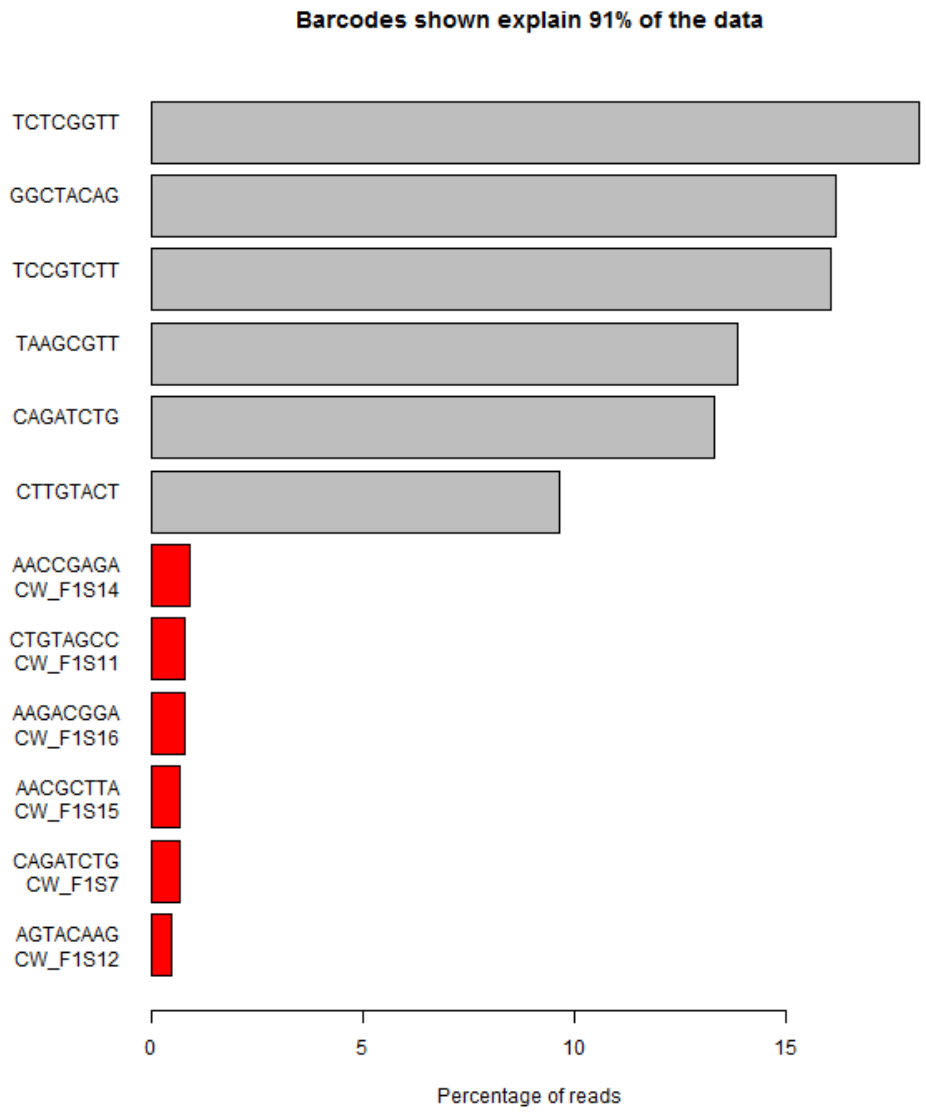
Tracking

Tracking - Barcodes



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Tracking

Tracking Exercise

You have some barcode statistics from a set of runs from the same group.

Red = Expected barcode
Grey = Unexpected barcode

Can you see if you can spot a problem within this data set,
and say how many of the lanes it might affect?

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Tracking

Tracking – Swapped Samples

- Swapped between users
  - Different sample type
  - Different species
  - See later Contamination / Biology sections
- Swapped within experiment
  - Look for consistent biological signal
  - Use other knowledge of the samples to validate

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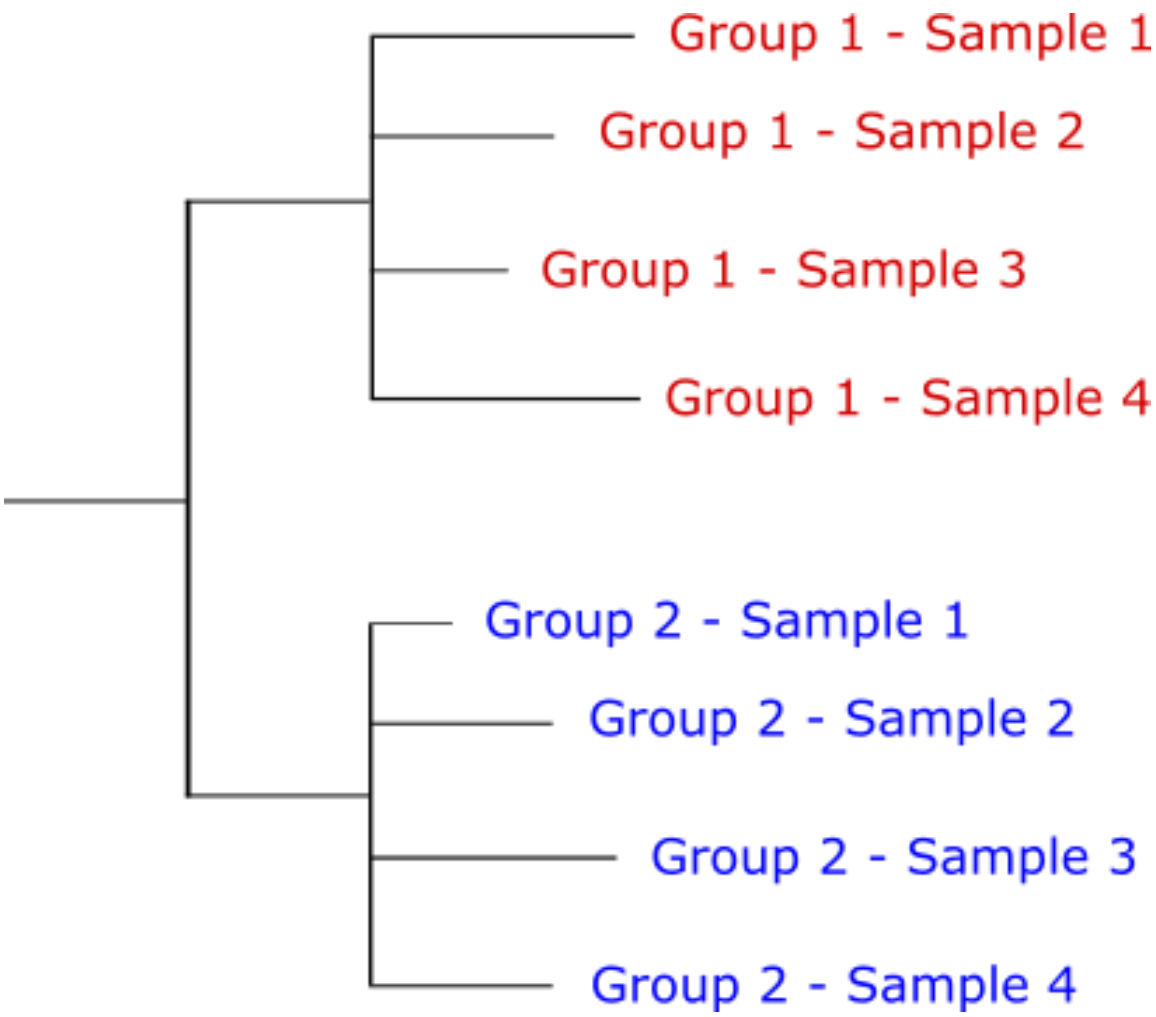
Tracking

Tracking – Sample groups



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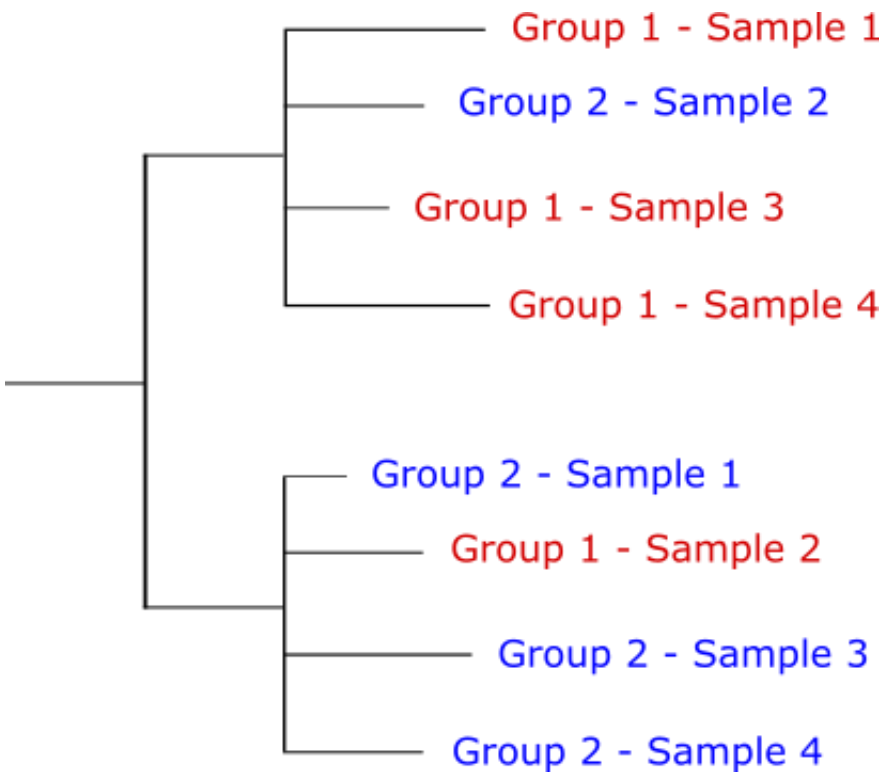
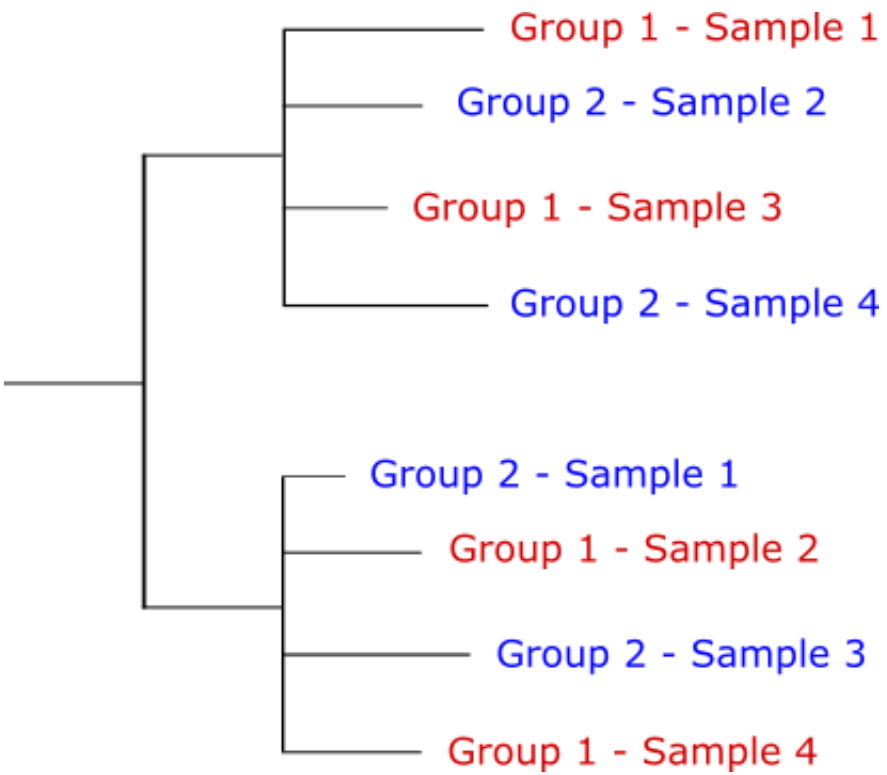
Tracking – Sample groups





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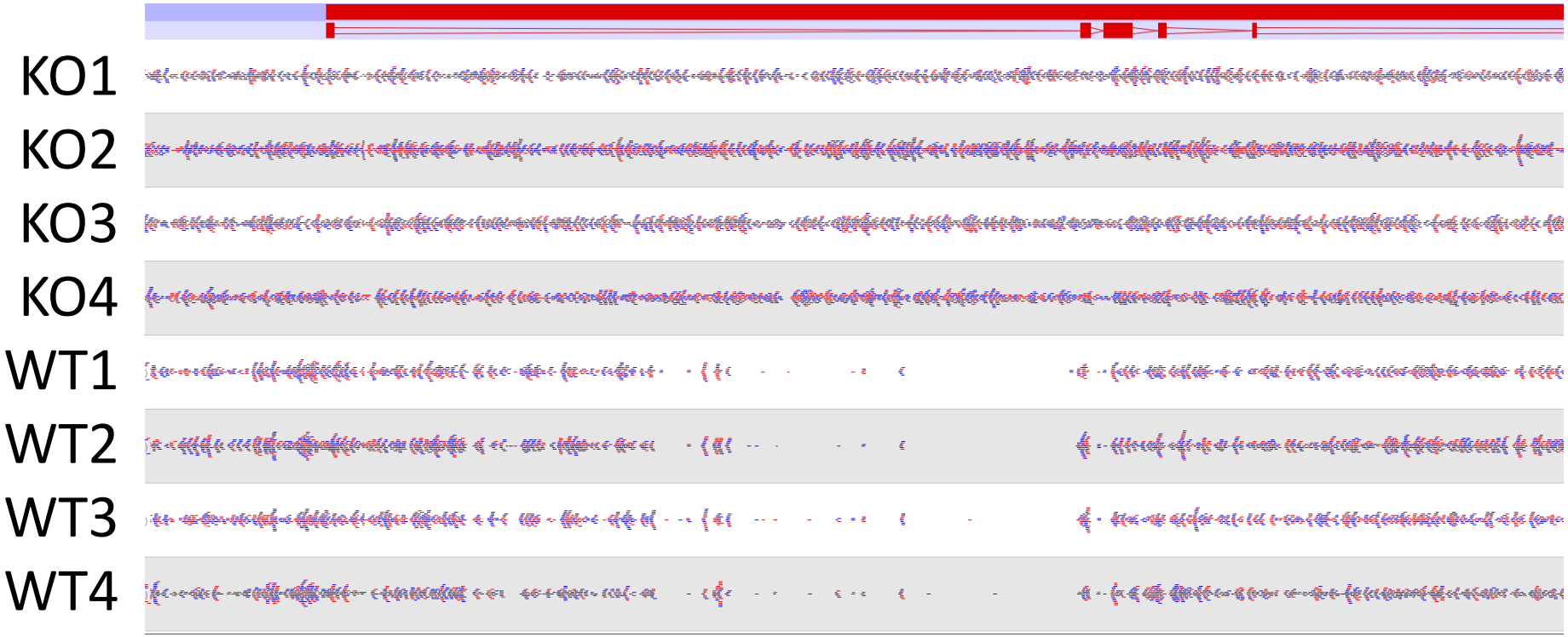
Tracking

Tracking – Sample swaps



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Library

Library Problems

- Material Lost
  - Overamplification
    - Duplication
- Biases in selection
  - Priming bias
  - GC bias
  - Methylation bias
  - Size selection bias
- Technical contamination
  - Read through adapter
  - Adapter dimers

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Library

Duplication

- Over-sequencing of library complexity
- Too little material or too much PCR
- Can be difficult to assess

- Why does duplication matter?
  - Potentially biased
  - Over-estimates measurement accuracy

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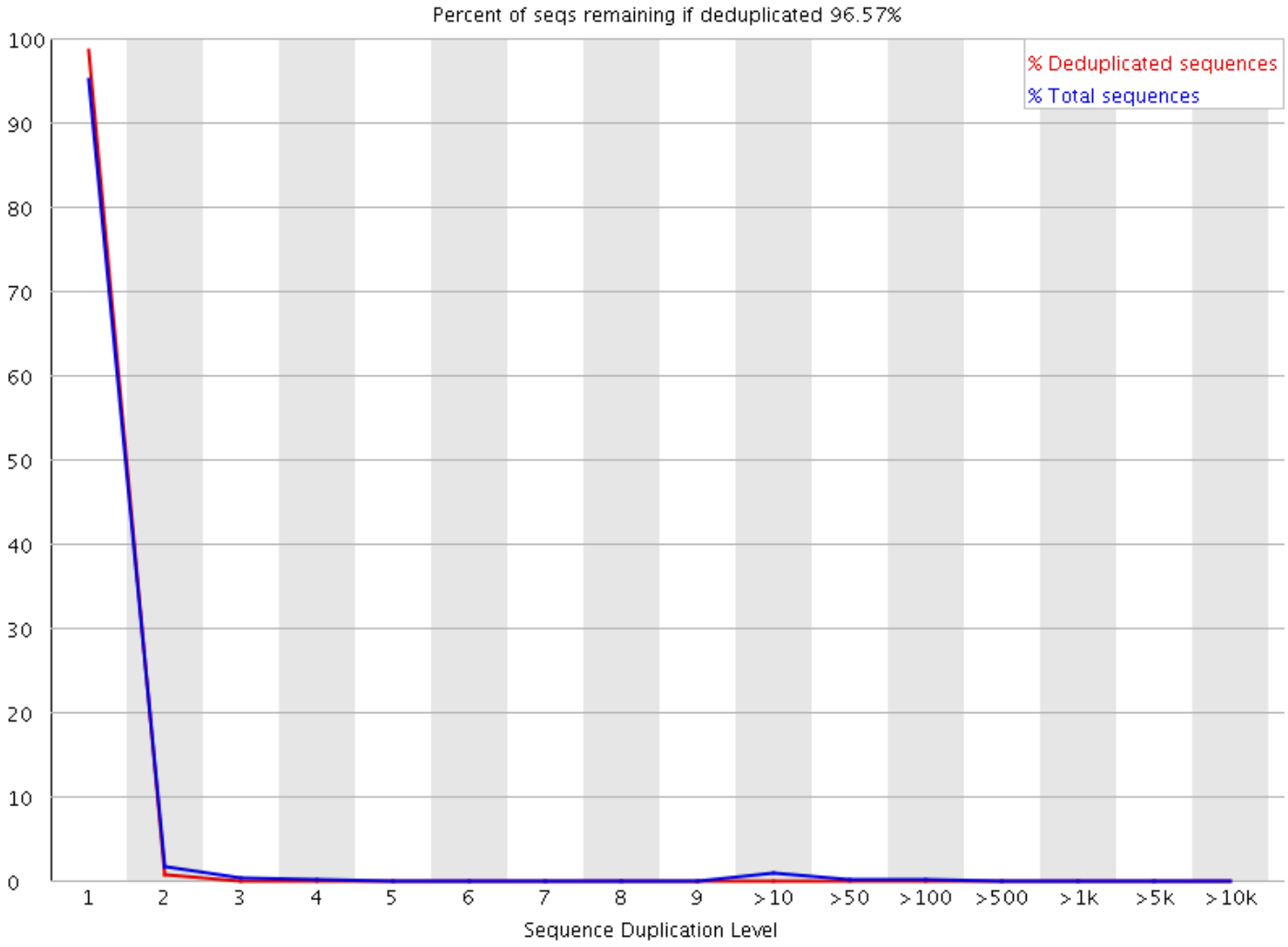
Library

Duplication



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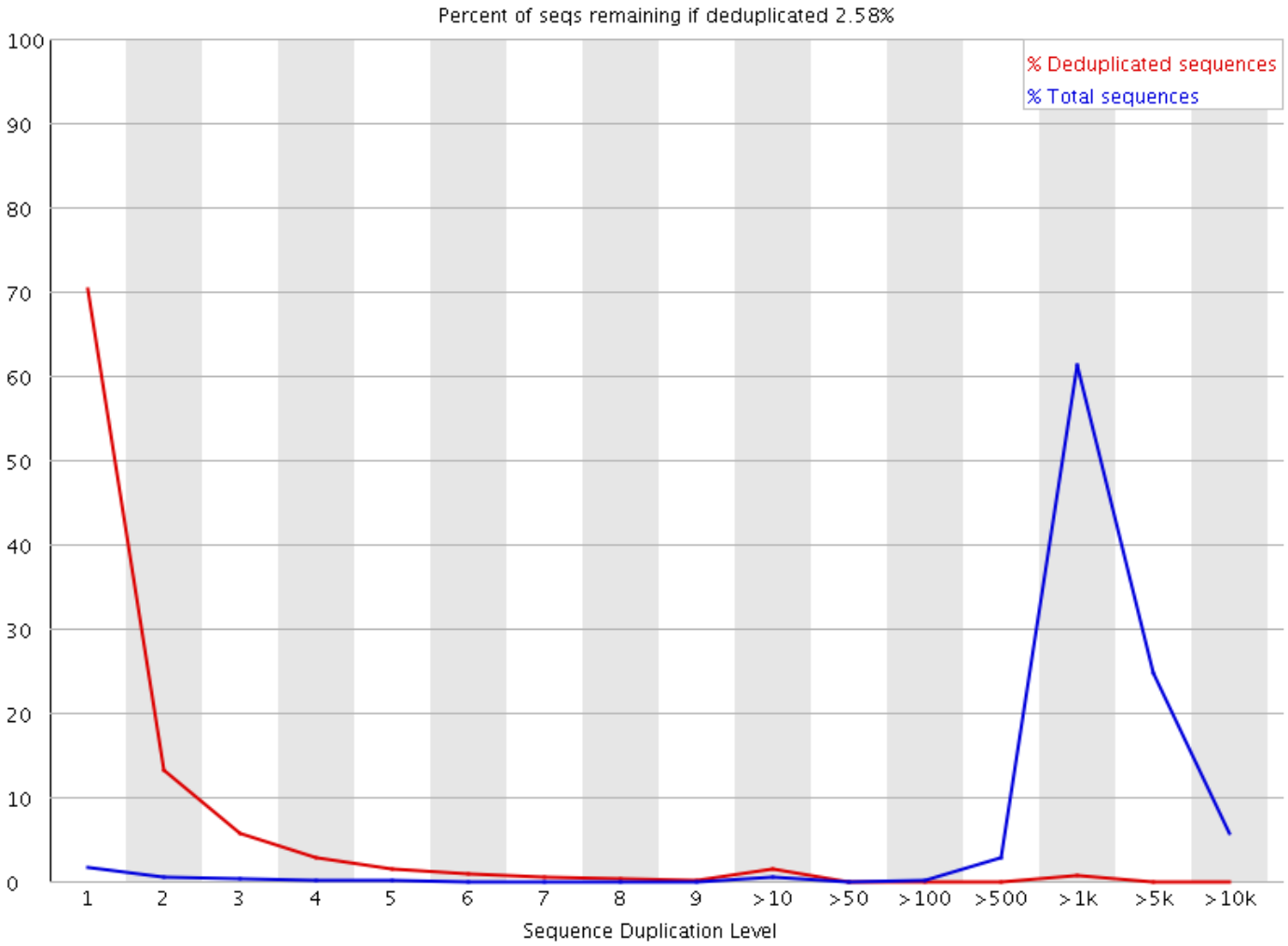
Library

Duplication



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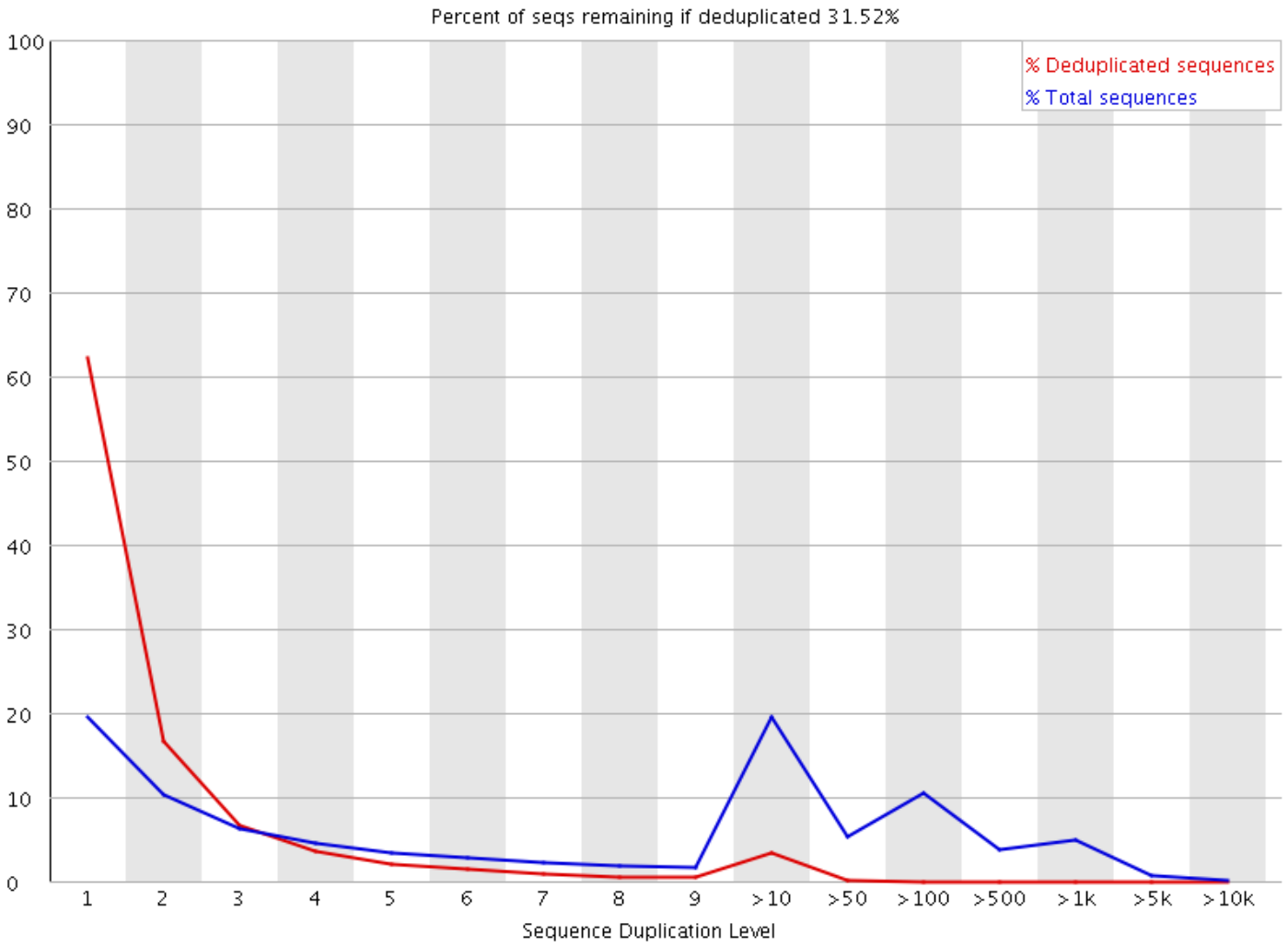
Library

Duplication



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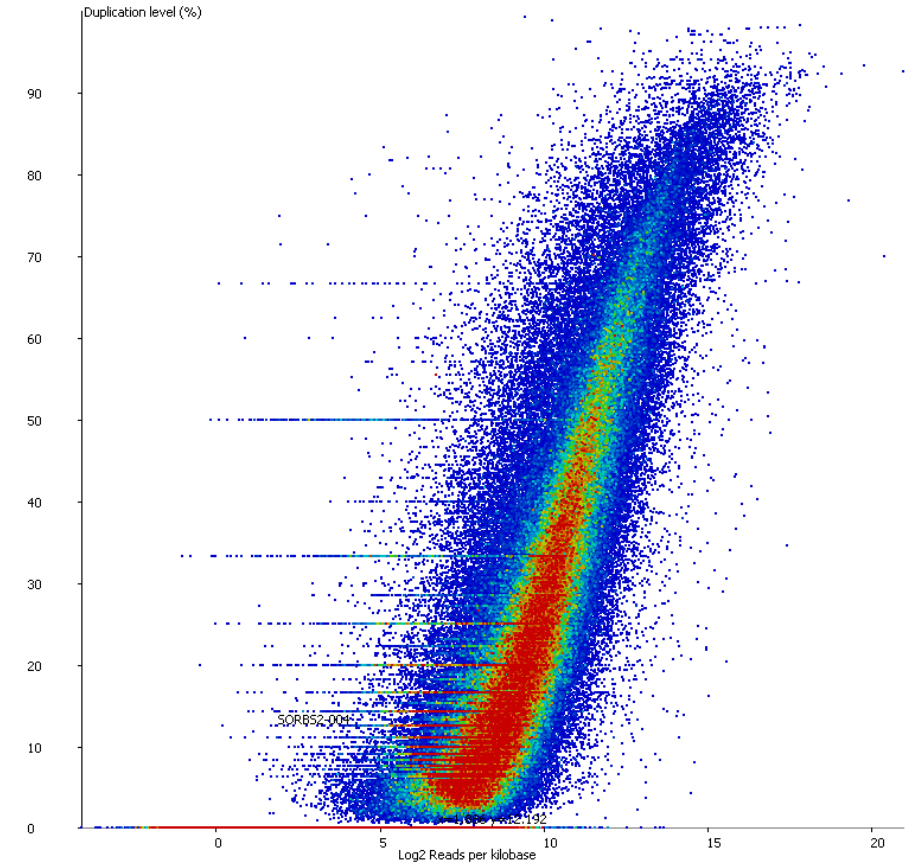
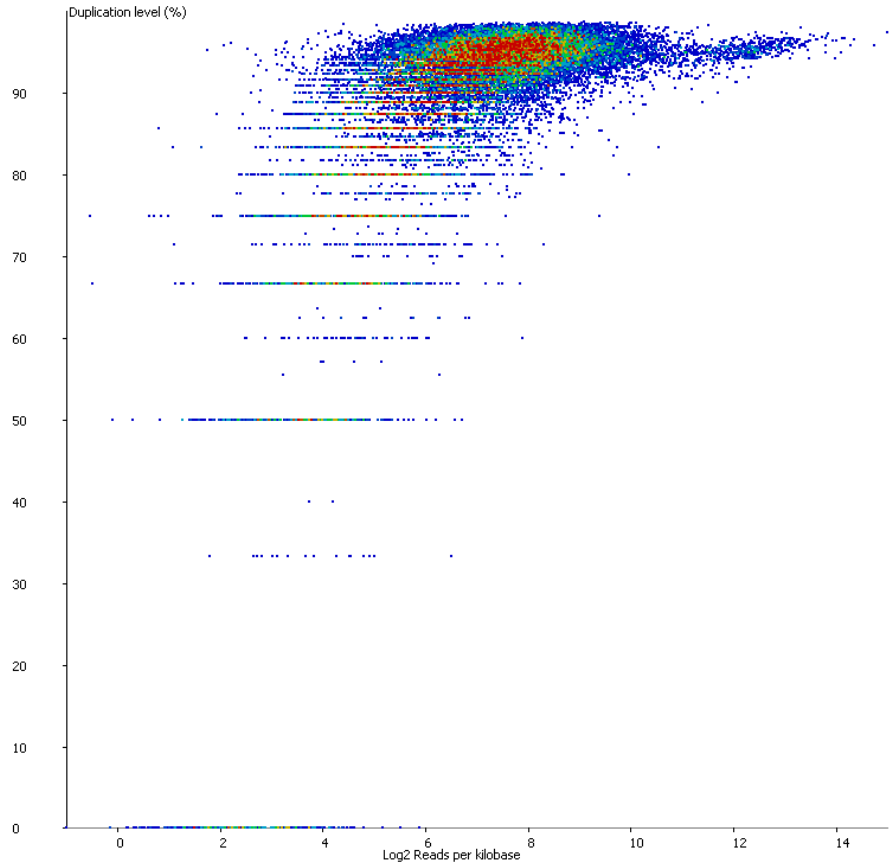
Library

Duplication





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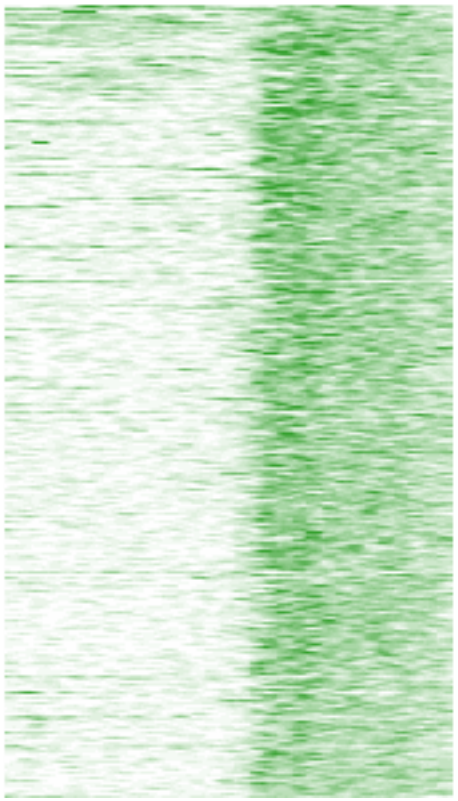
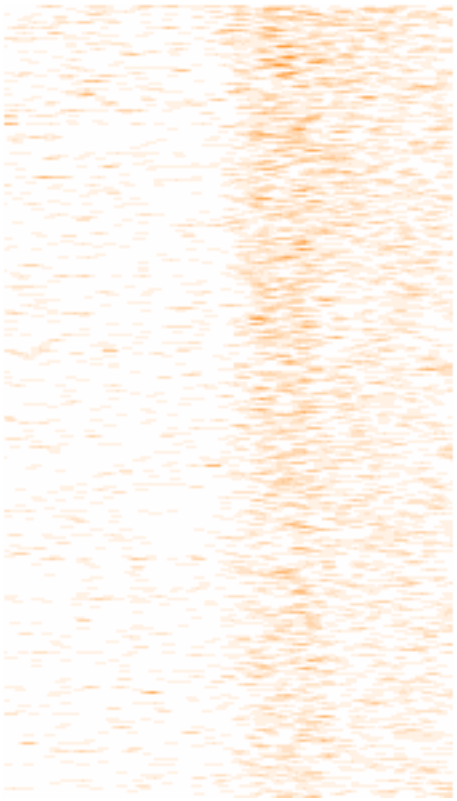
Library

Duplication - Repeats





Repeat

Repeat

Real

Mapped

Deduplicated

Peak callers (MACS for example) deduplicate internally, so you don't have to consciously do this.

Only avoided by using uniquely mapped reads.

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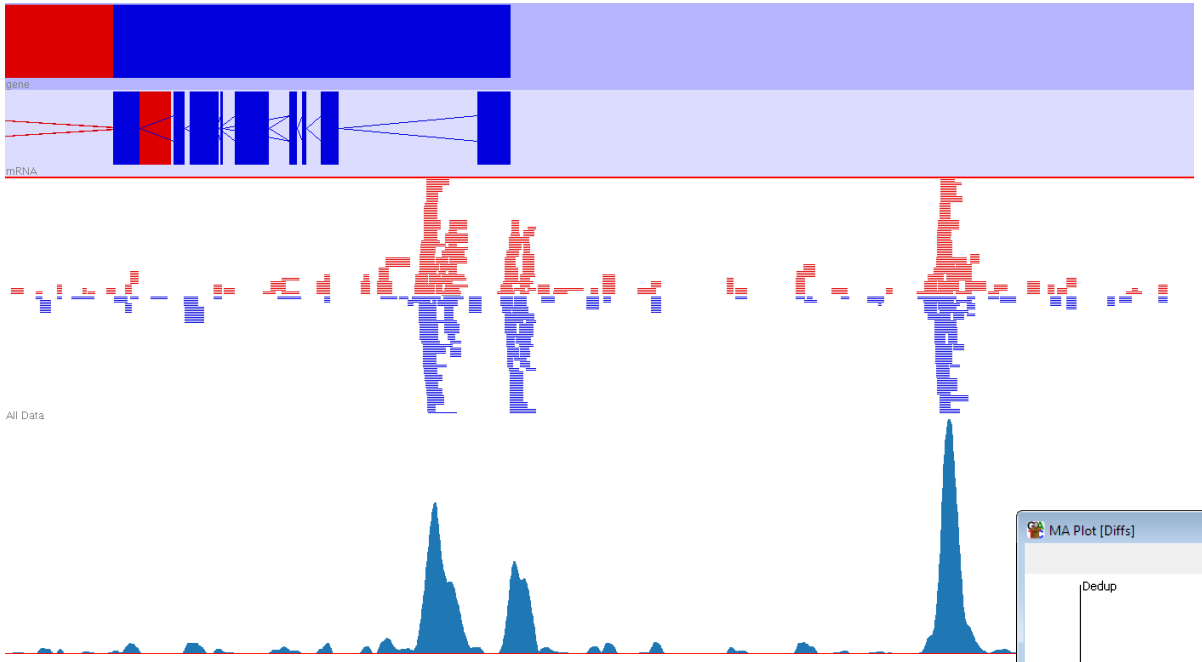
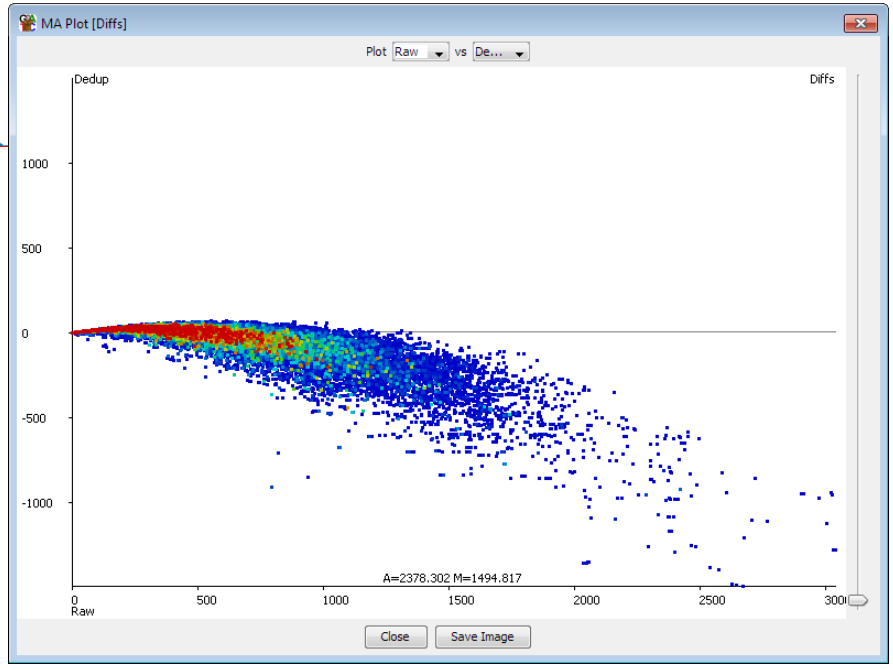
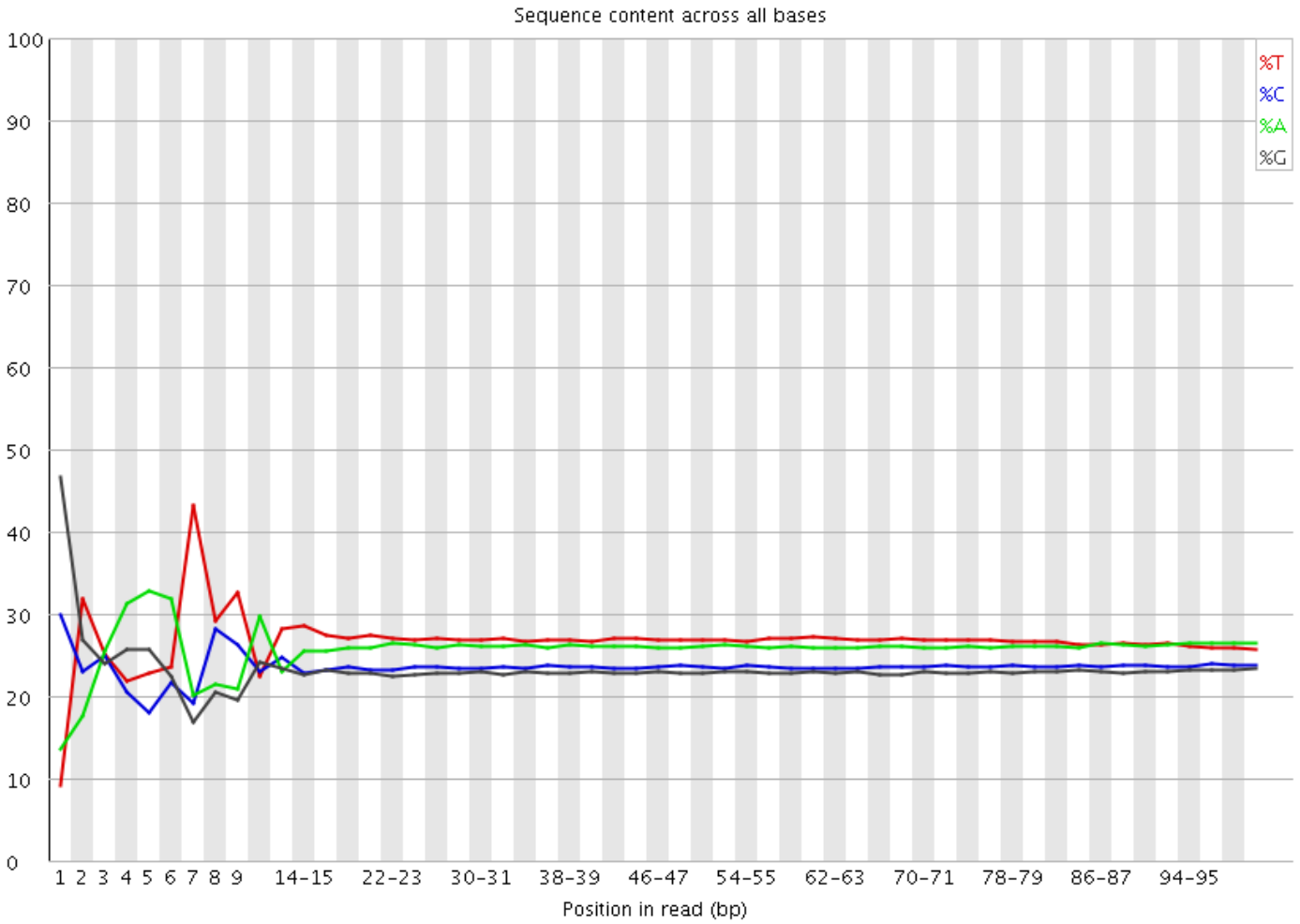
Deduplication





Library

Priming Bias



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Contamination

Contamination

Contamination

- Different kinds
  - Technical contamination
    - Adapter dimers
  - Contamination with a species you might expect
    - *E.coli* in a mouse sample
  - Contamination with something unexpected
  - Contamination with the wrong material
    - DNA in an RNA-Prep
  - Mixed samples

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Contamination

Mapping Efficiency

- Know what to expect
  - Data type (genomic / transcriptomic)
  - How good / complete is the genome

- Distinguish unique / multi-mapped reads
  - Understand the mapping process

```
Reads:
                Input:    5725730
               Mapped:    4703342 (82.1% of input)
            of these:     471516 (10.0%) have multiple  alignments
                                 (471516 have >1)
82.1% overall read alignment rate.
```

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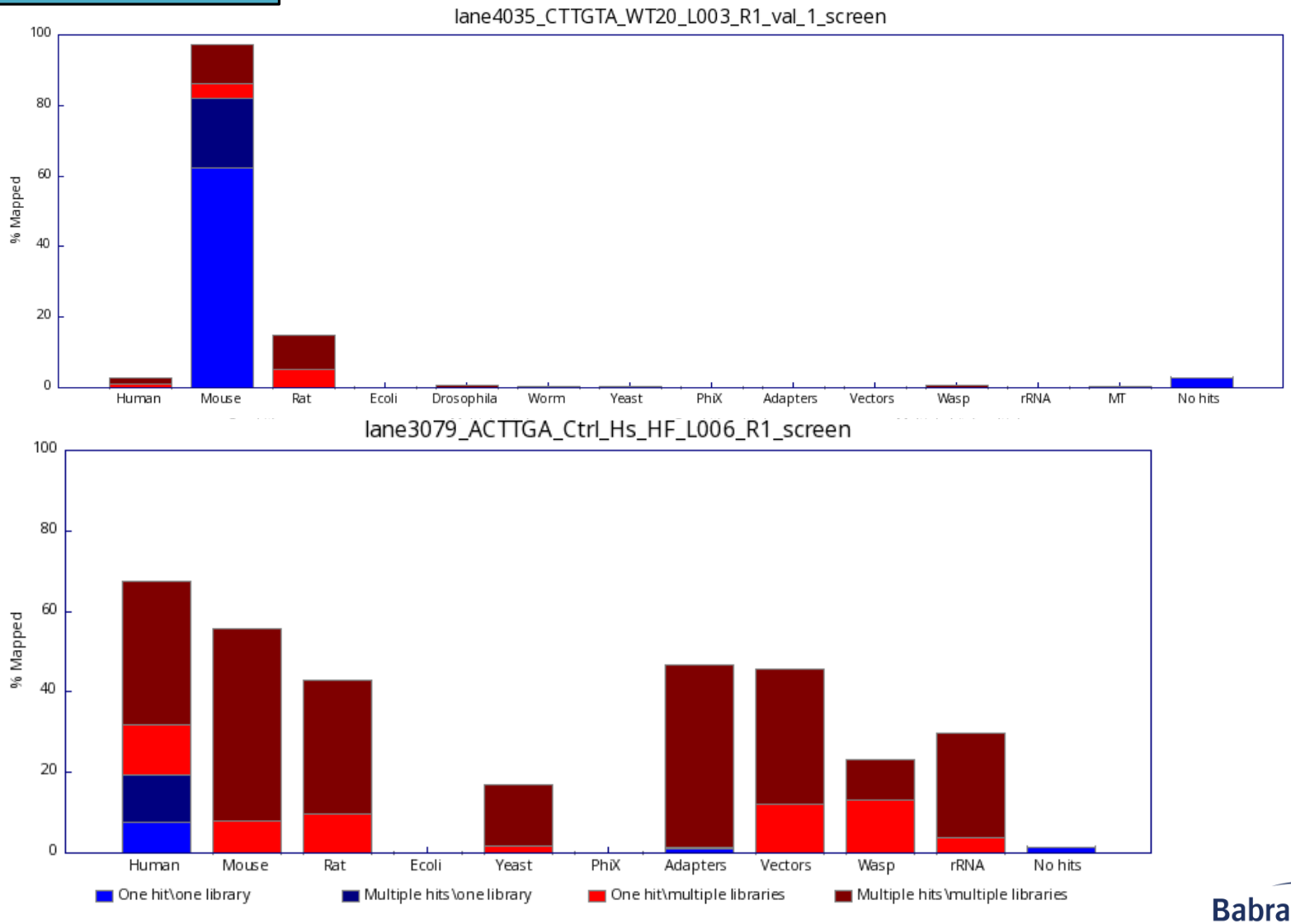
Contamination

Species Screen



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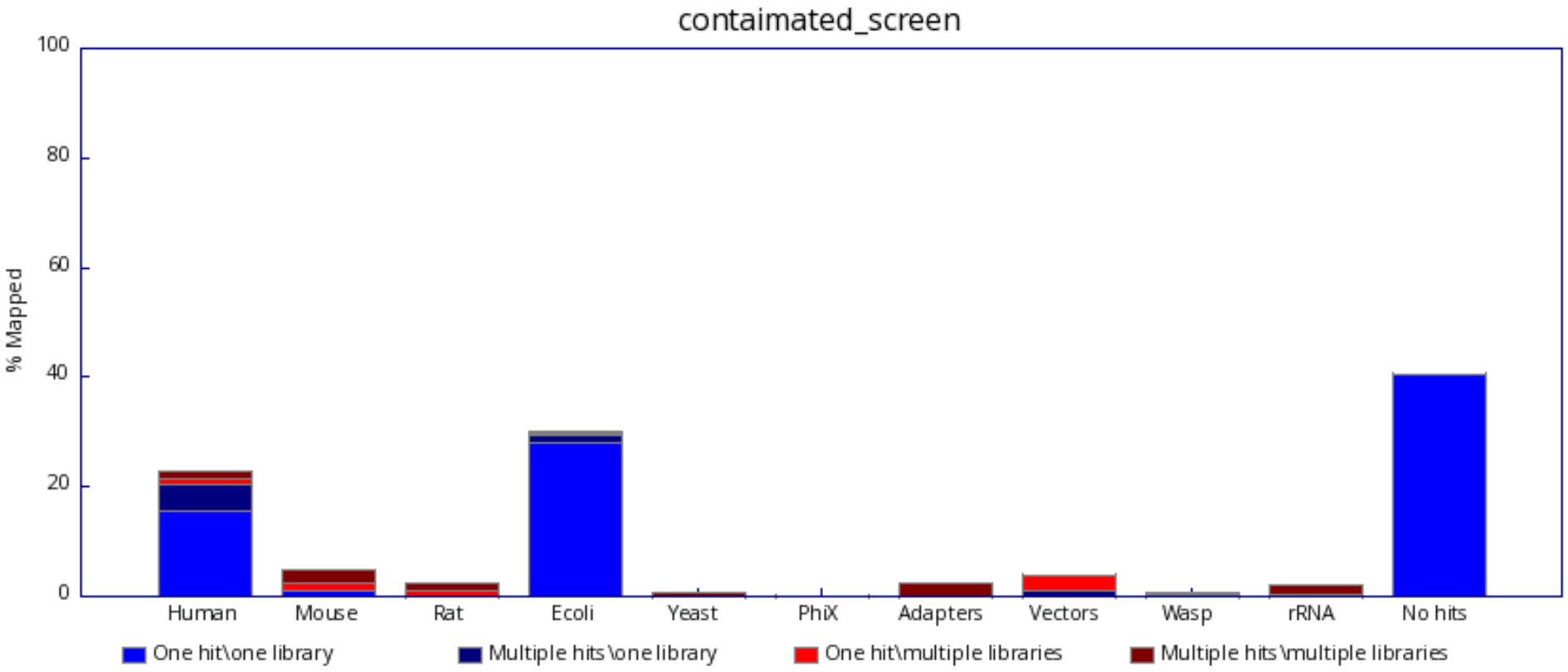
Contamination

Species Screen



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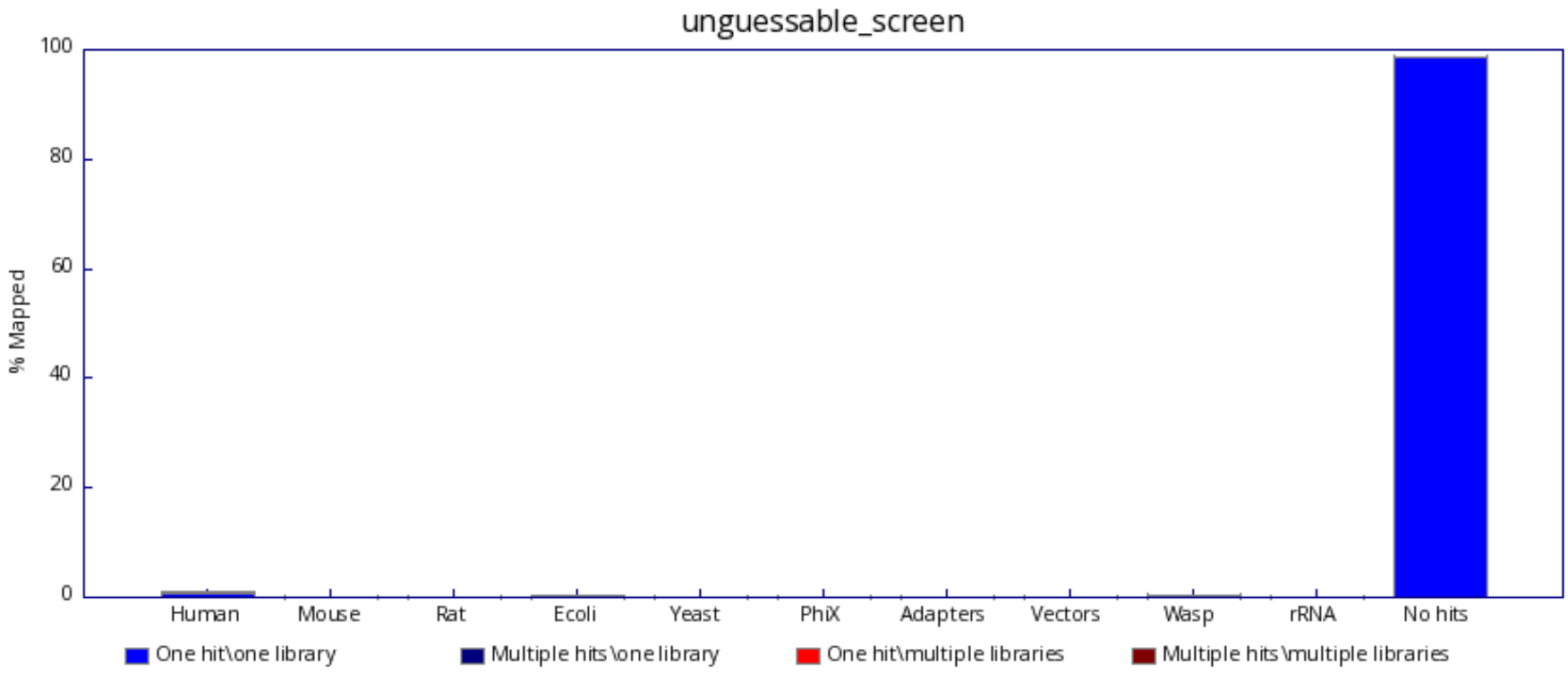
Contamination

Species Screen



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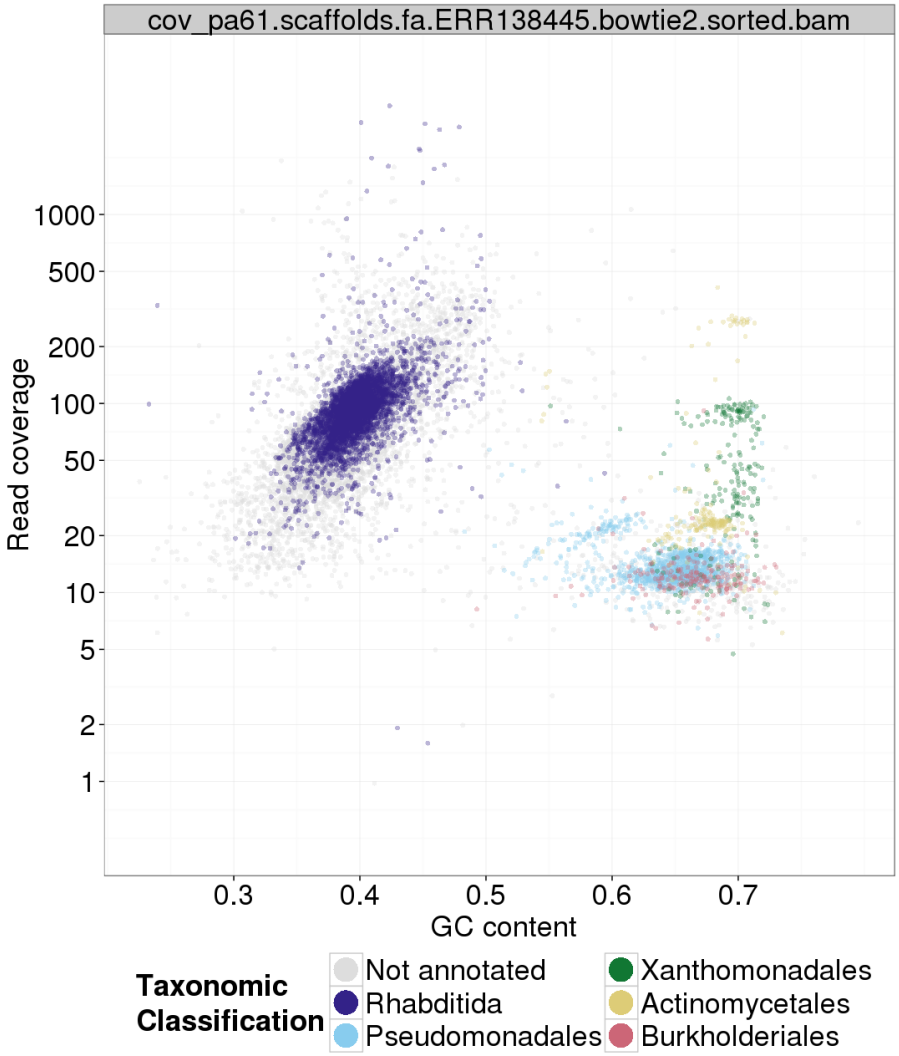
Contamination

TAGC Plots



Assemble

↓

Filter contigs

↓

Plot %GC vs Coverage

↓

Sample and blast

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Contamination

Contamination

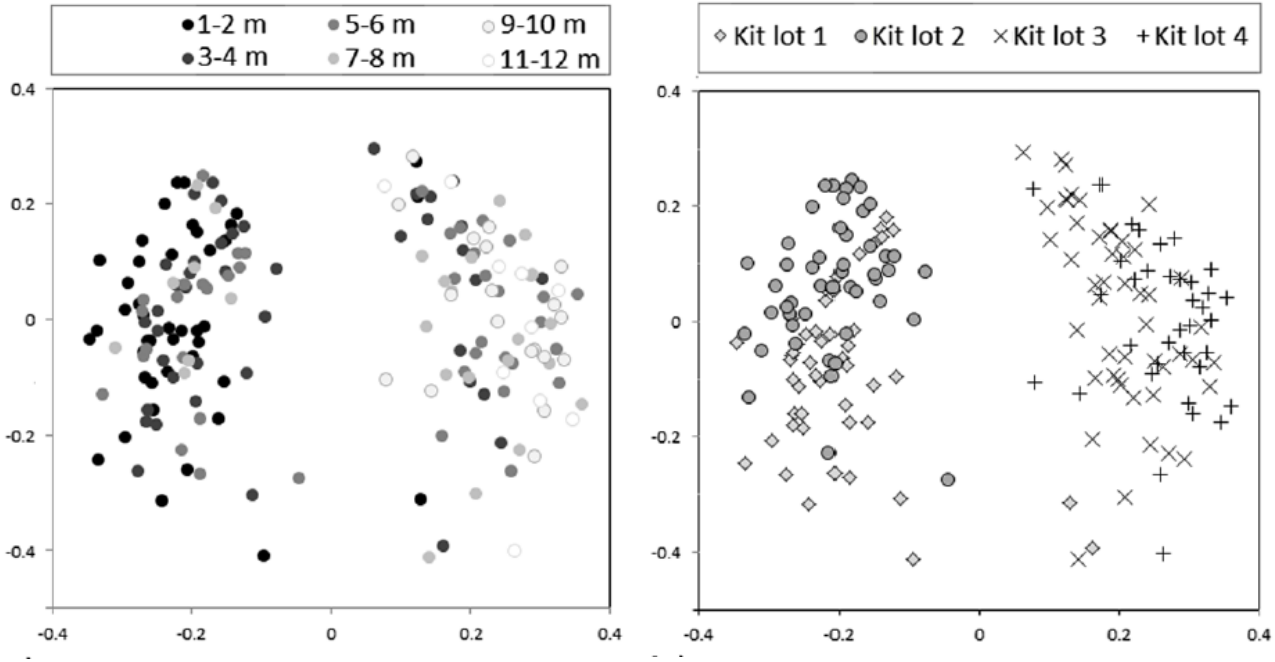
Salter *et al. BMC Biology* 2014, **12**:87
http://www.biomedcentral.com/1741-7007/12/87

BMC Biology

RESEARCH ARTICLE Open Access

Reagent and laboratory contamination can critically impact sequence-based microbiome analyses

Susannah J Salter[1*], Michael J Cox[2], Elena M Turek[2], Szymon T Calus[3], William O Cookson[2], Miriam F Moffatt[2], Paul Turner[4,5], Julian Parkhill[1], Nicholas J Loman[3] and Alan W Walker[1,6*]



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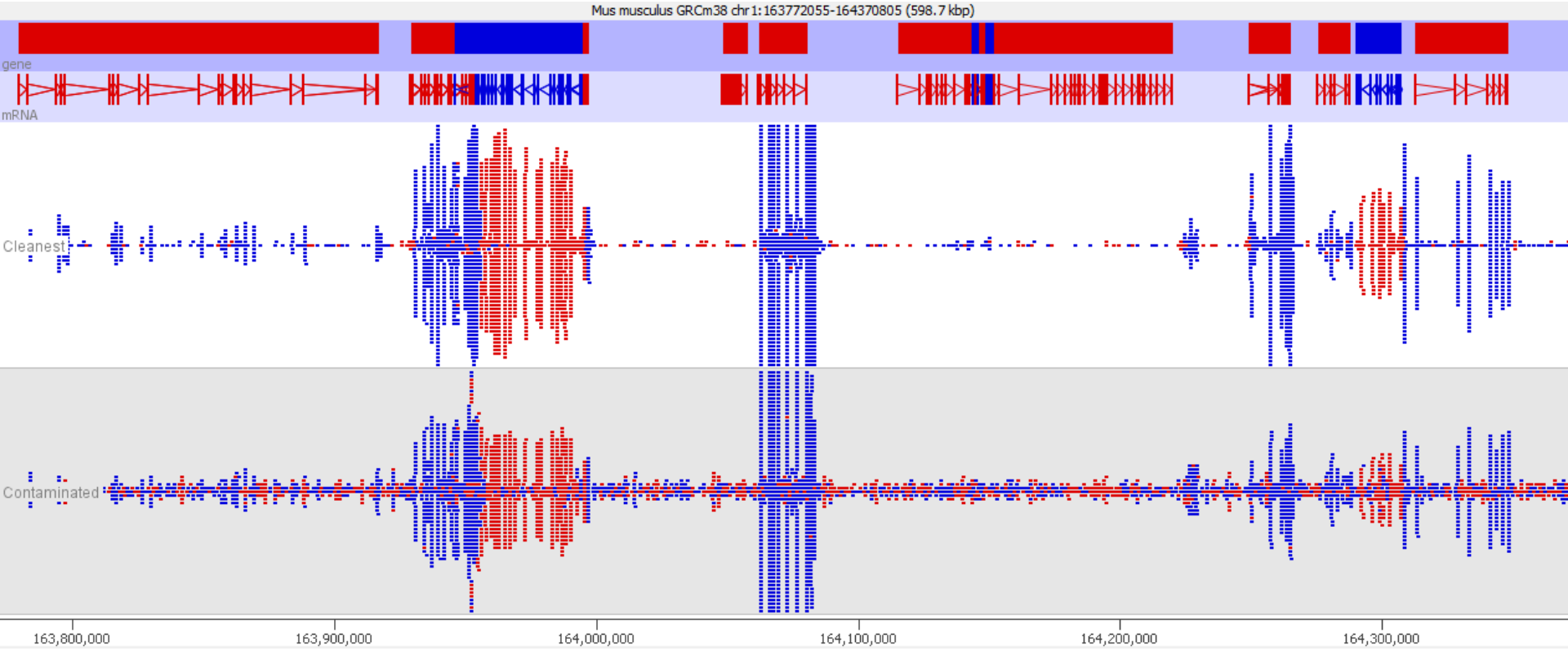
Contamination

Internal Contamination



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Biological

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Biological

Samples Don't Behave

- All samples come with a set of expectations
  - Biological effect
  - Sample source
  - Rough biological behaviour
- If these aren't met
  - Samples may not be what you expect
  - Statistical analyses may be invalid
  - Larger biological picture may be missed

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Biological

Exercise

You have been given a set of QC and visualisation results for a knockout in male black6 mice (same genotype as the reference) of a single gene.

Have a look through the plots and see if there is anything which would cause you concern regarding the behaviour of the samples.

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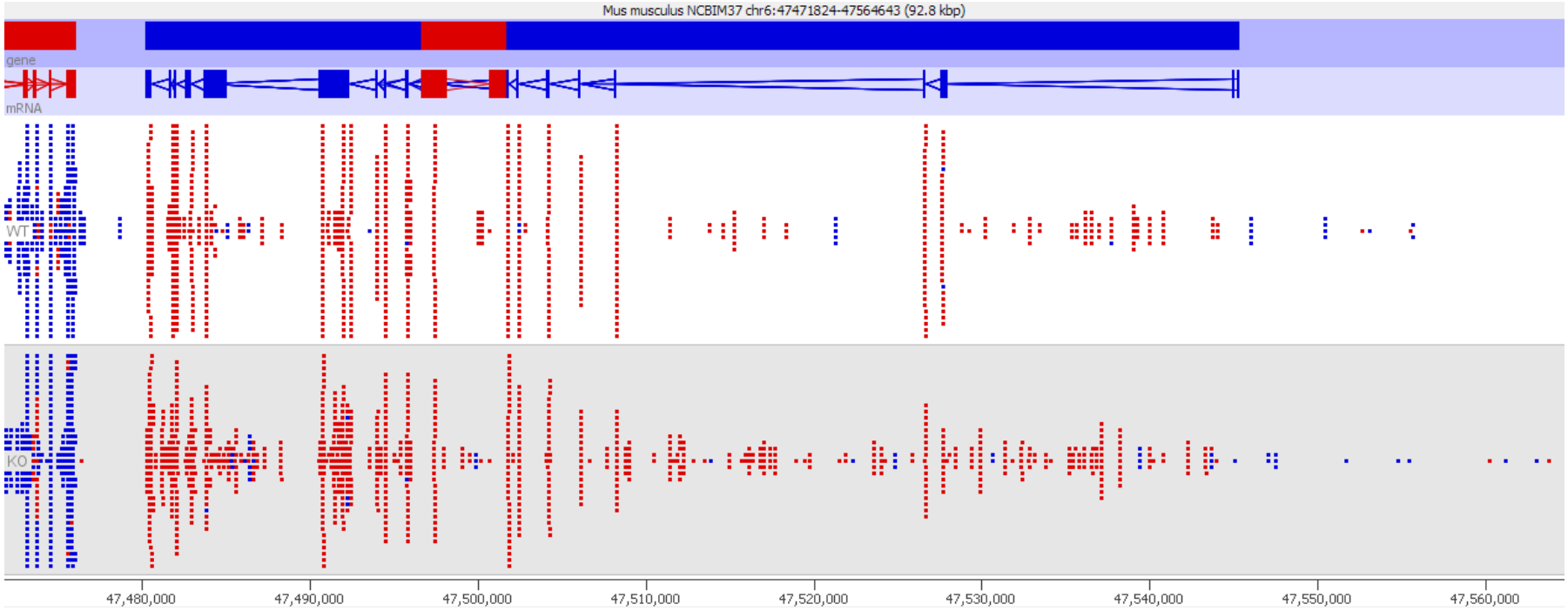
Biological

Expected effects missing



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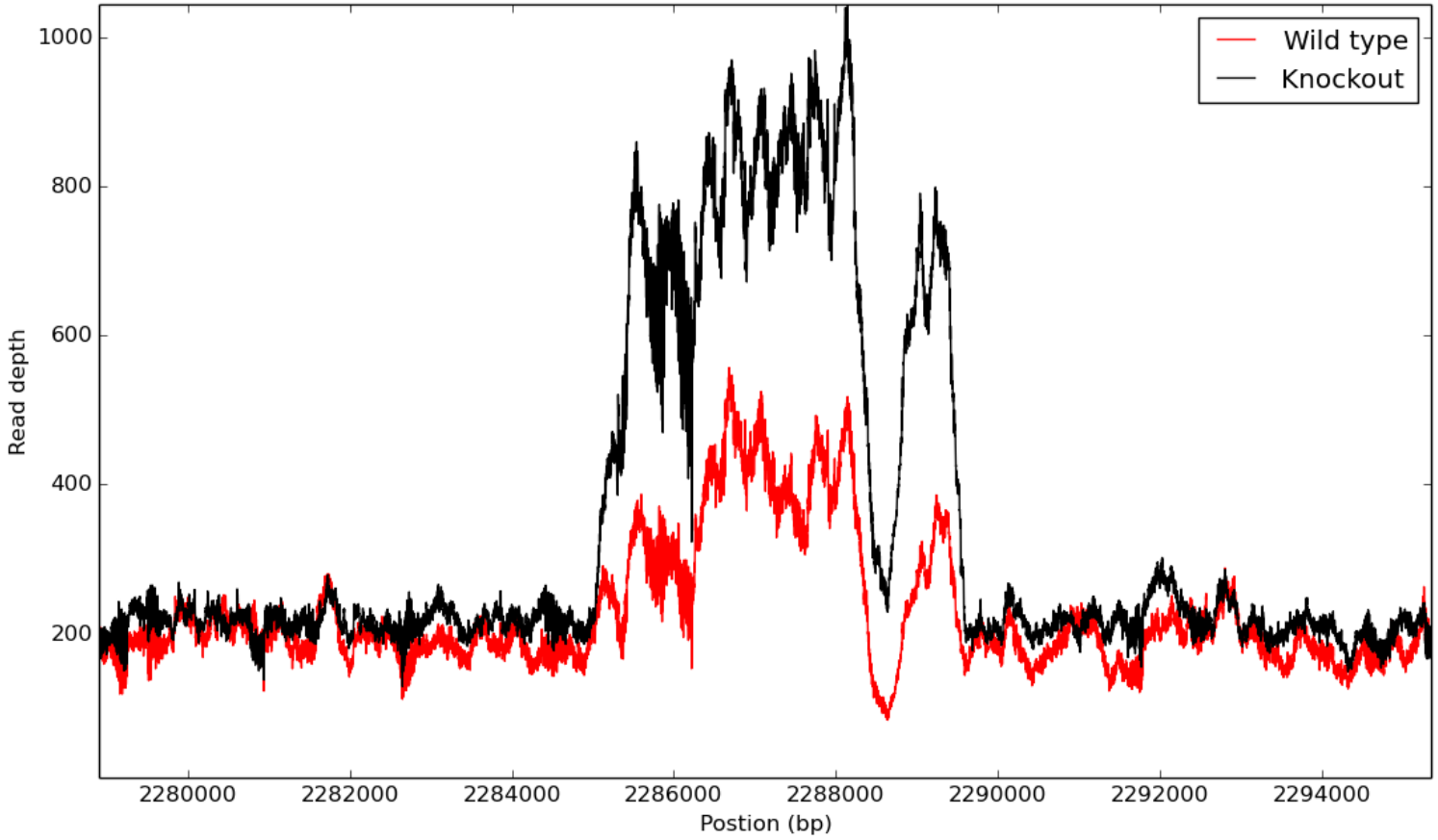
Confounded effects



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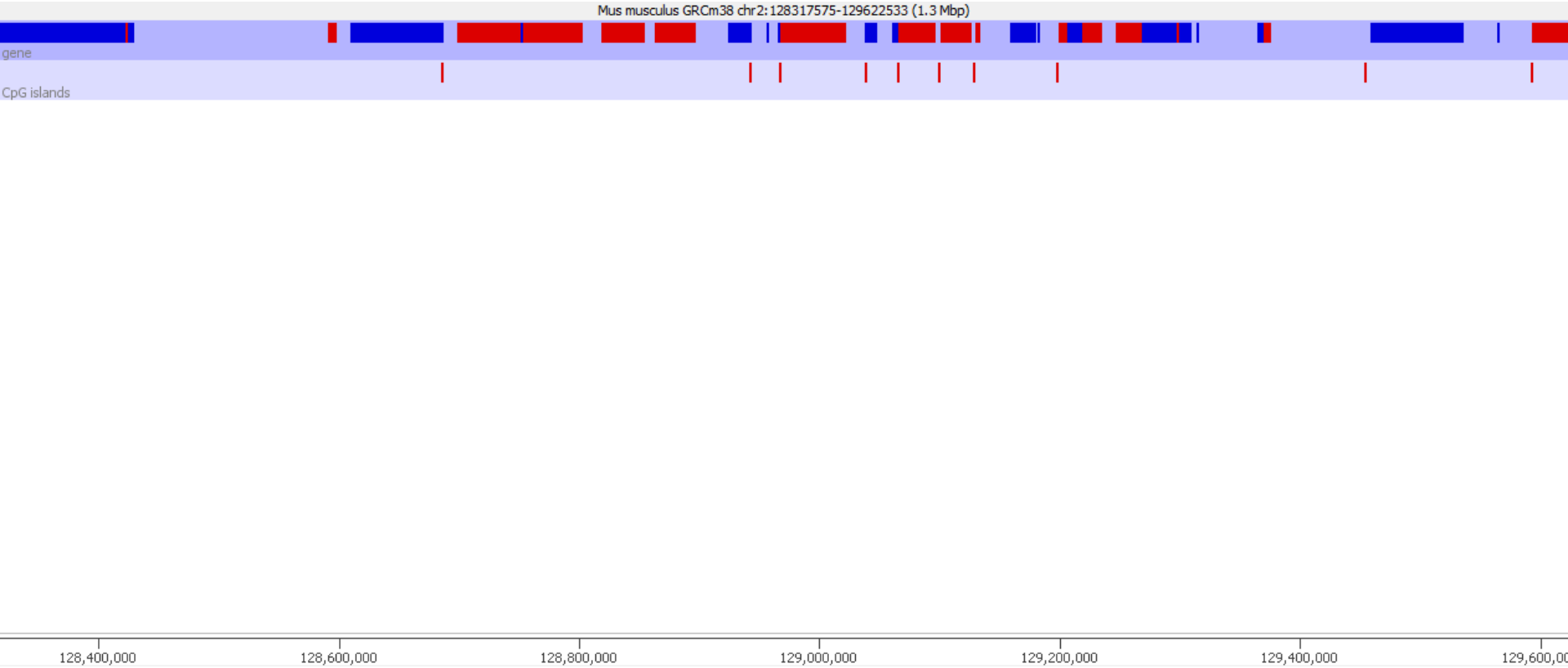
Biological

ChIP doesn't behave



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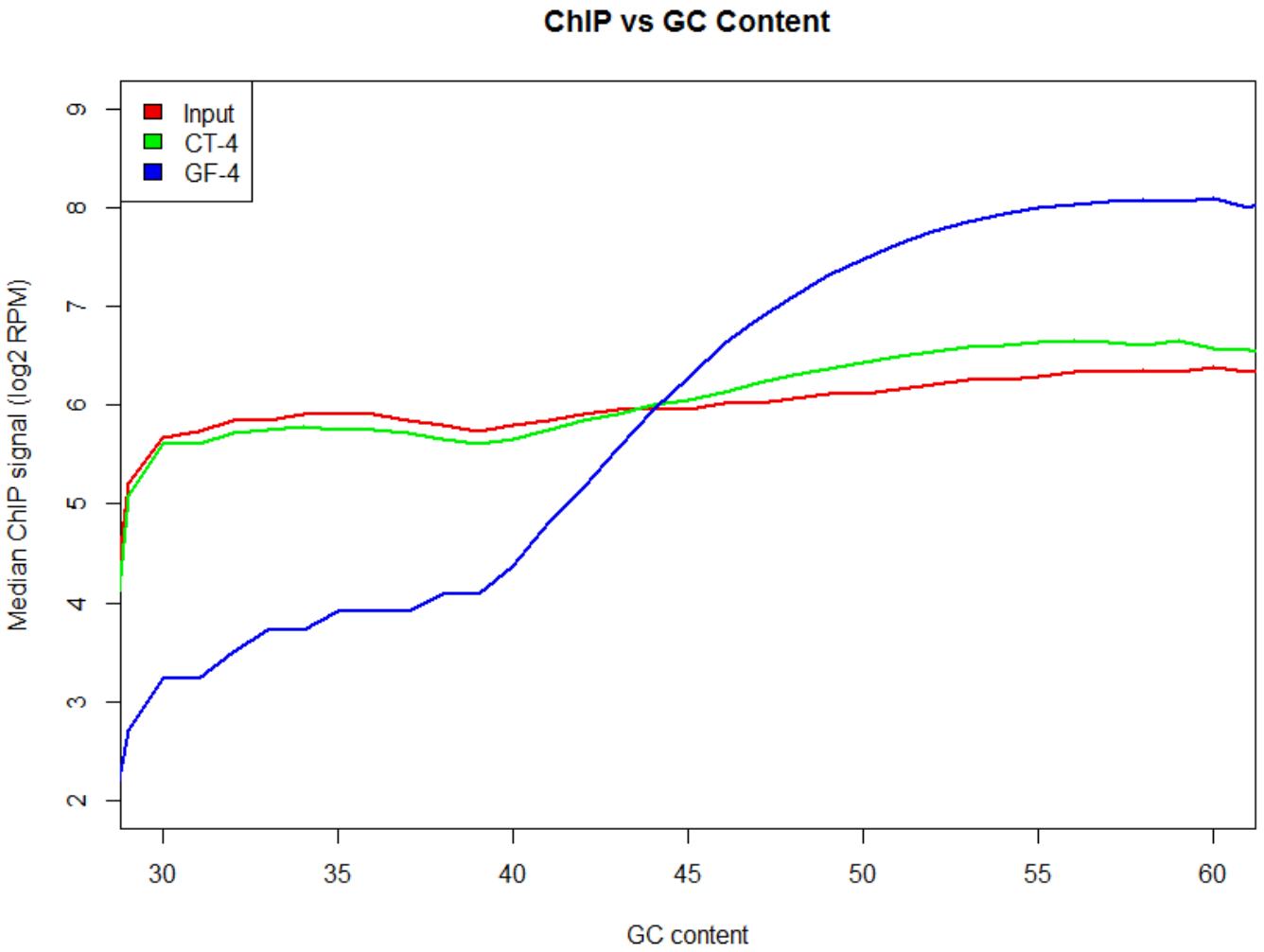
ChIP doesn't behave



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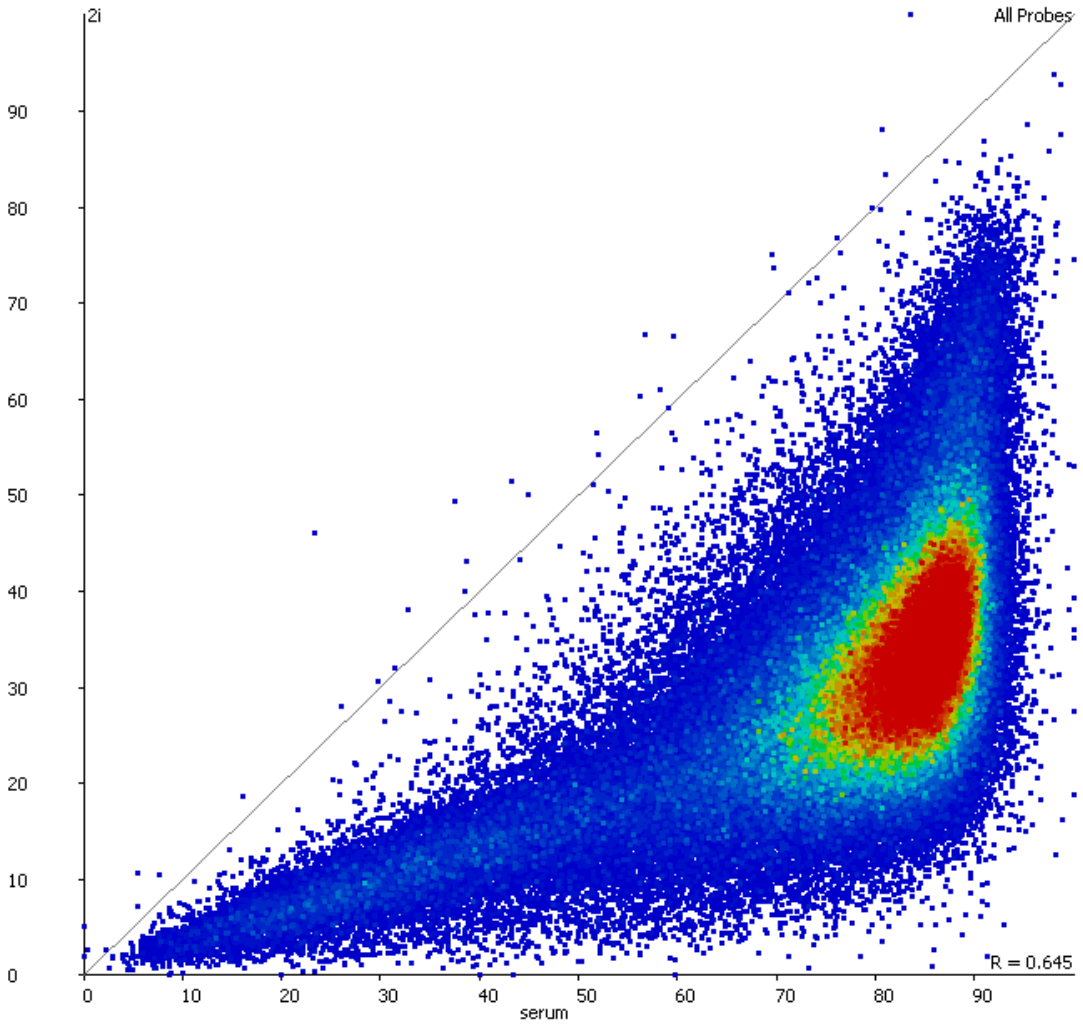
Biological

Methylation doesn't behave



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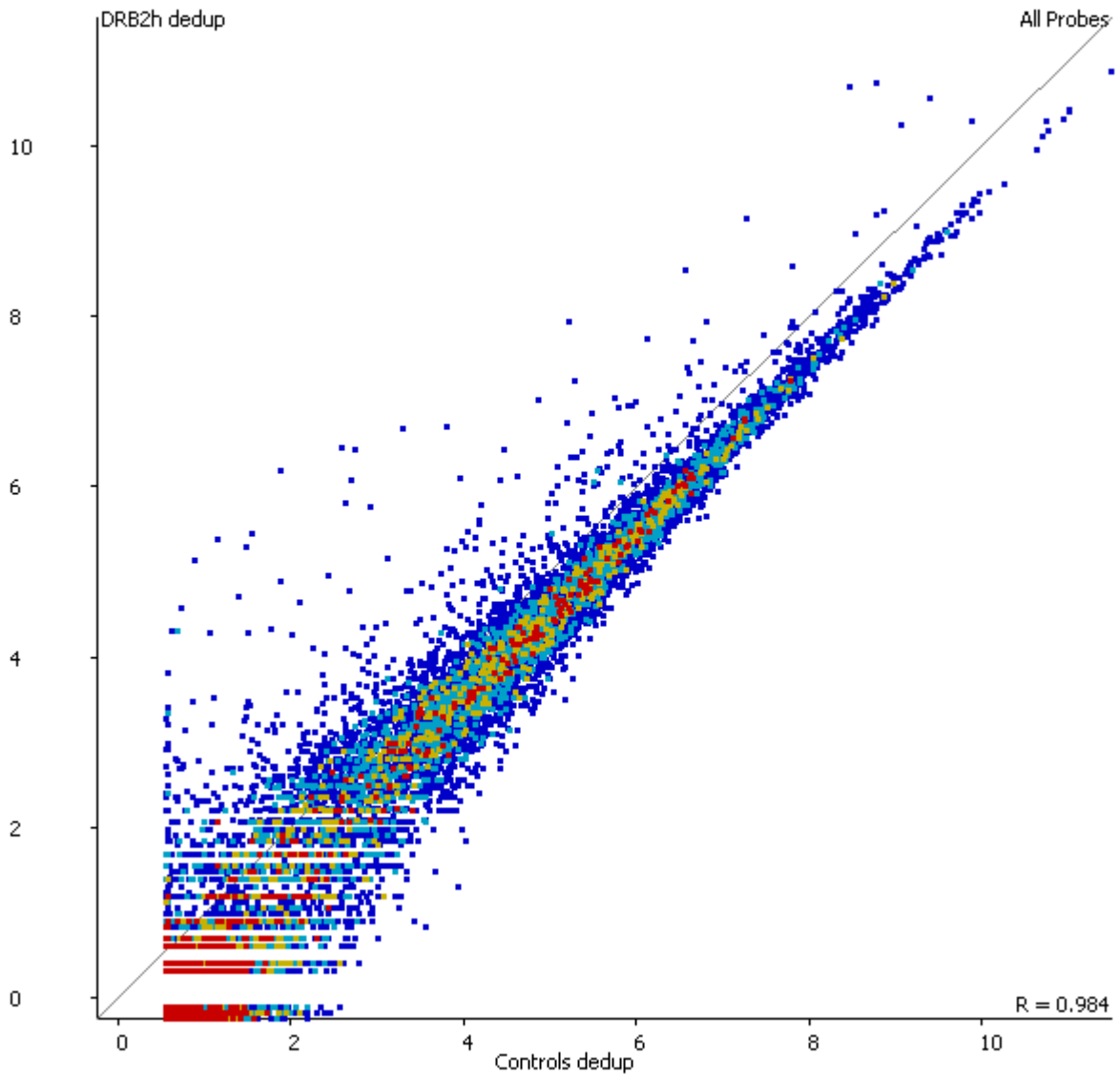
Biological

RNA-Seq doesn't behave



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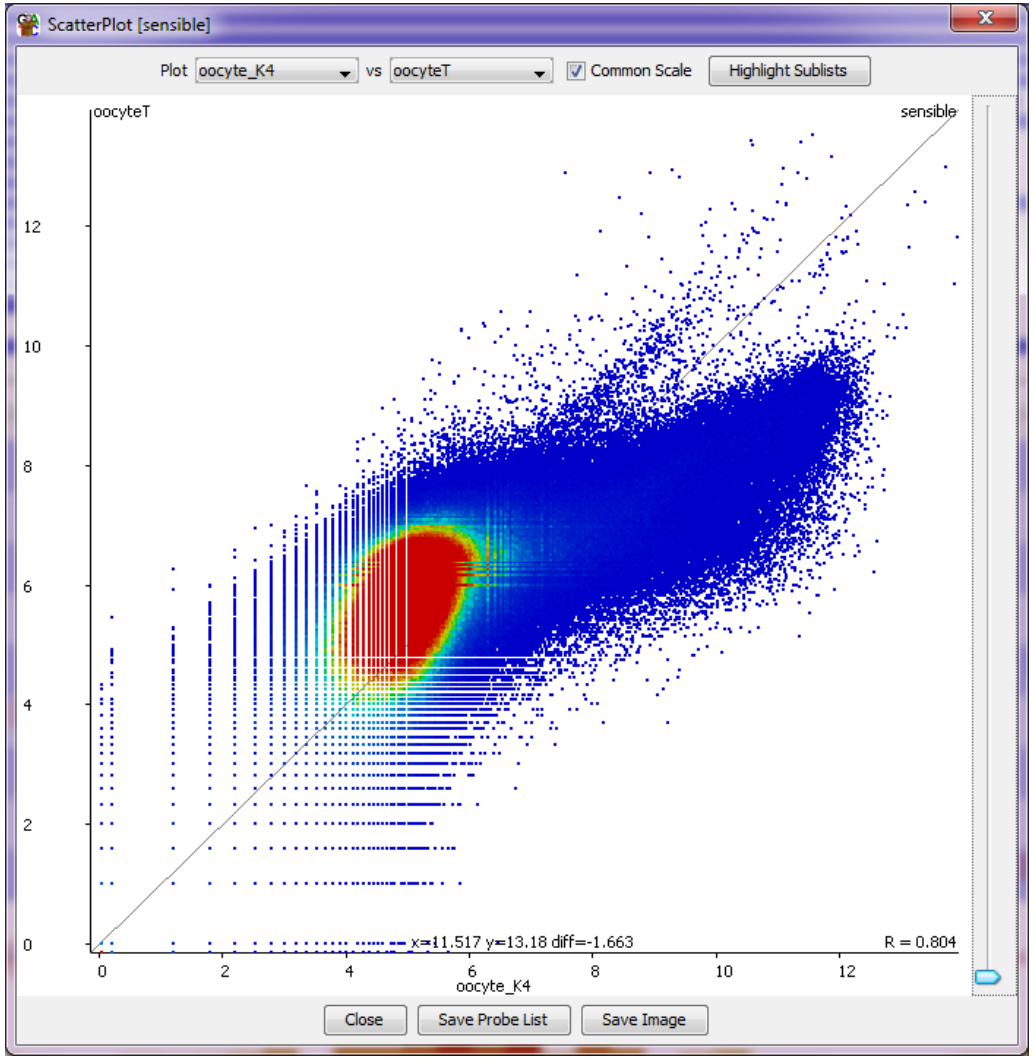
Biological

Multiple subgroups



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Interpretation

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