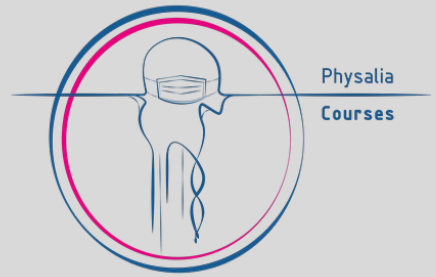
Processing NGS data

Epigenomics Data Analysis

Jacques Serizay

Physalia 2023



NGS processing workflow

- Get .bcl files
- Create fastq files
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- Filter duplicates, artifacts, …
- Generate tracks
- Assay-specific downstream analysis

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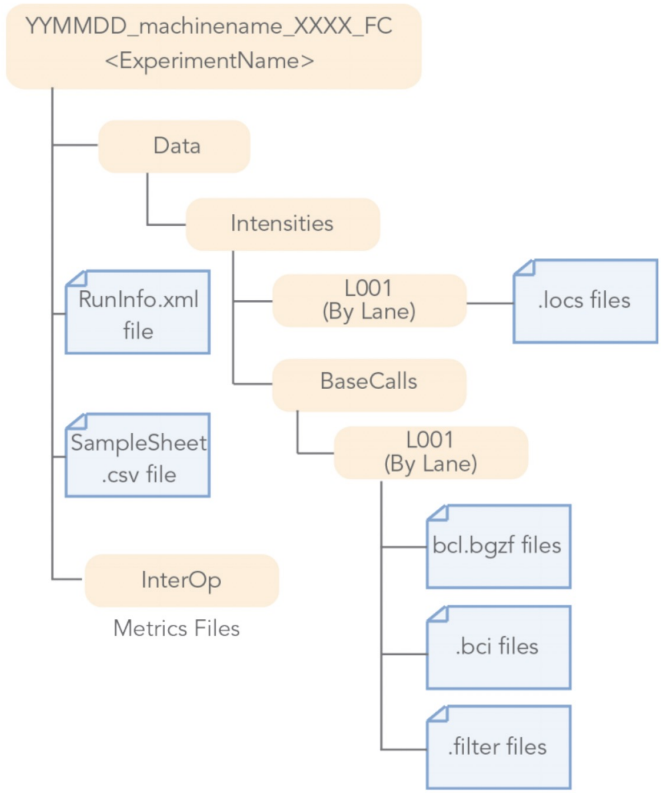
.bcl files

.bcl:

- **Raw data** output of a sequencing run
- **Binary**, non-human-readable file

- Contains the **base calling** and **quality score per cluster, per sequencing lane, per cycle**

- **Huge files**
- **No aggregated sequence per read**



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Fastq files

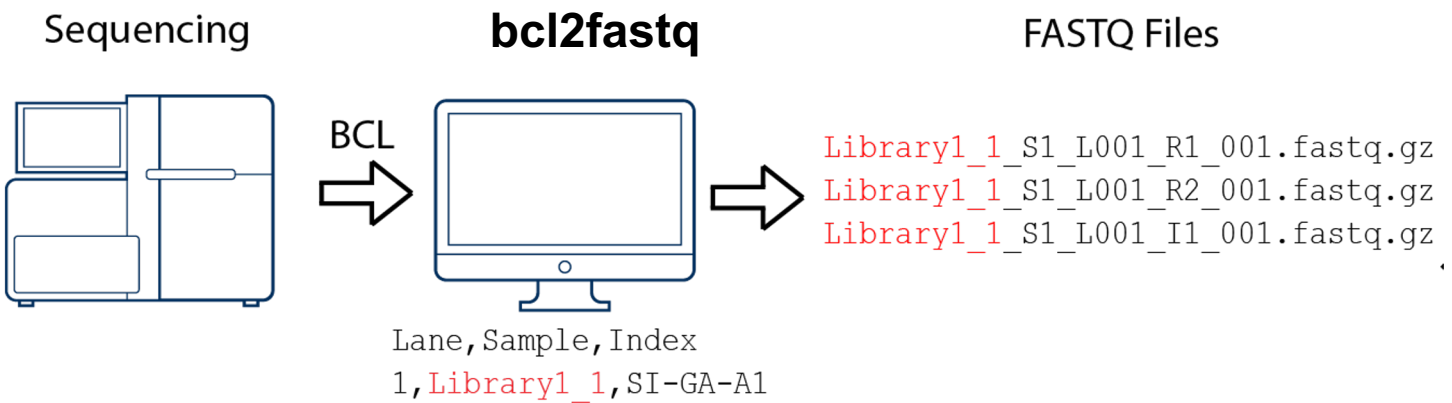
A fastq file contains reads, each read is composed of 4 lines:

1. A sequence identifier with information about the sequencing run
2. The sequence (the base calls; A, C, T, G and N).
3. A separator, which is simply a plus (+) sign.
4. The base call quality scores, using ASCII characters to represent the numerical quality scores.

```
jacquesserizay@LOCAL[12:46:19]:~ $ cat SRR11575369_1.fastq.gz | zcat |  head -n 8
@SRR11575369.1 1/1
ANCAACAGTGGAATTCGTTTTGATGAAAAAAATAAATTTGTTCTTCAAAGCAGAGTGAATGATGCAGTACGAGCTCTTGCTCTTGAAAACCCATCACAACTTTATAATTTAATAATTAGTGAAAAATTAAAAAAATAATATTTCTTATATT
+
F#F:FFFFF:FFFFFFFFFF:F,FF,FF::F,::FFFFFFFFFFF:FF:FF:F,FFF,F:F,FFF,FF:FF:F:FFF:FF:FFFF,:F::FFFF:FF,FF:F:FF,,:F:F,::,F:F:FF,,::,FF:,,F,,,,,:FFFF::F:,,,FF
@SRR11575369.2 2/1
TNGCCAGTCAATACCGCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTGTTTTTTTTTTTTATATAATAAAATTTTTTTTTTTTTAATAAAATTTTTTTTTTATTTT
+
F#FFFFFFF::FF:FF::FFFFFFFFFFFFFFFF:FFFFFFFFFFFFF,,FFFFFFFFFF:FFFFFF:FFFFFFFFFFF:F,FFFFF,,,:,,::FFFFFFFF,,:,,,F,,,,,,FFFFFF,F,,FF:,F,:,F,FFFFF,,,:,,F::F
```

bcl2fastq

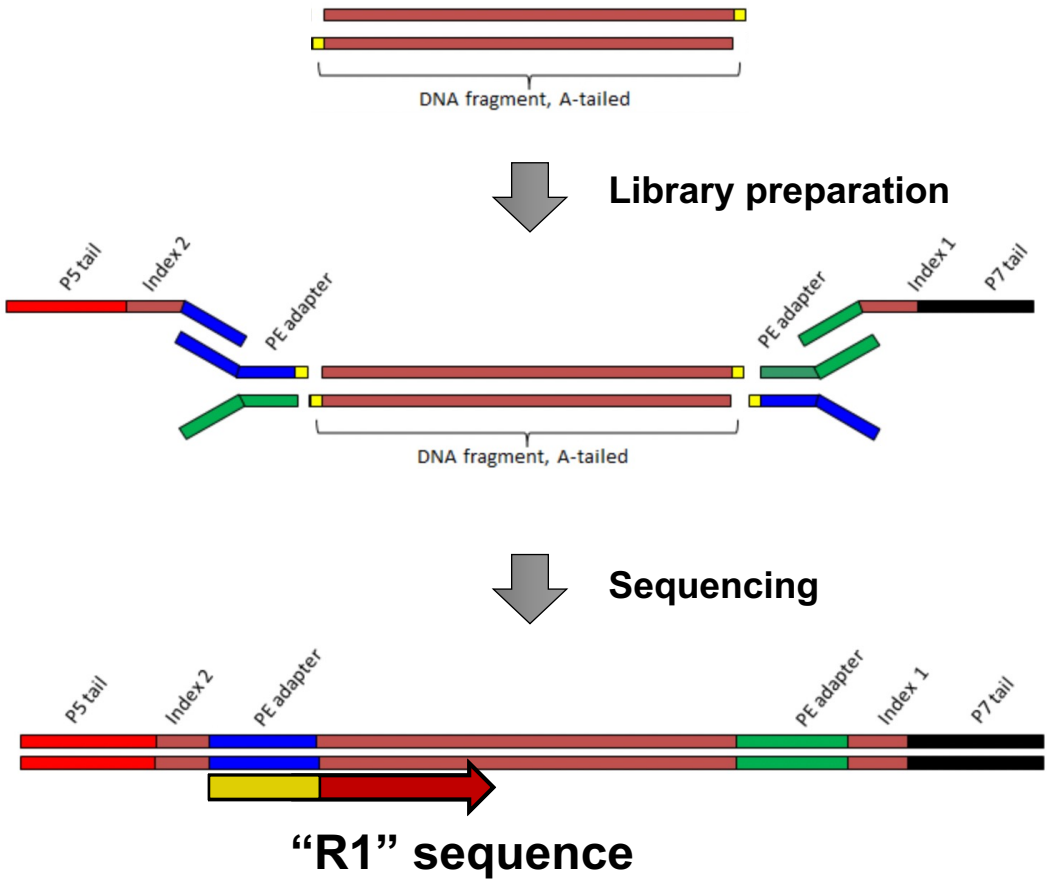
```
bcl2fastq --run-folder-dir <bcl_files_folder> --output-dir <fastq_files_folder>
```



User guide:

https://support.illumina.com/content/dam/illumina-support/documents/documentation/software_documentation/bcl2fastq/bcl2fastq_letterbooklet_15038058brpmi.pdf

Why so many fastq files?



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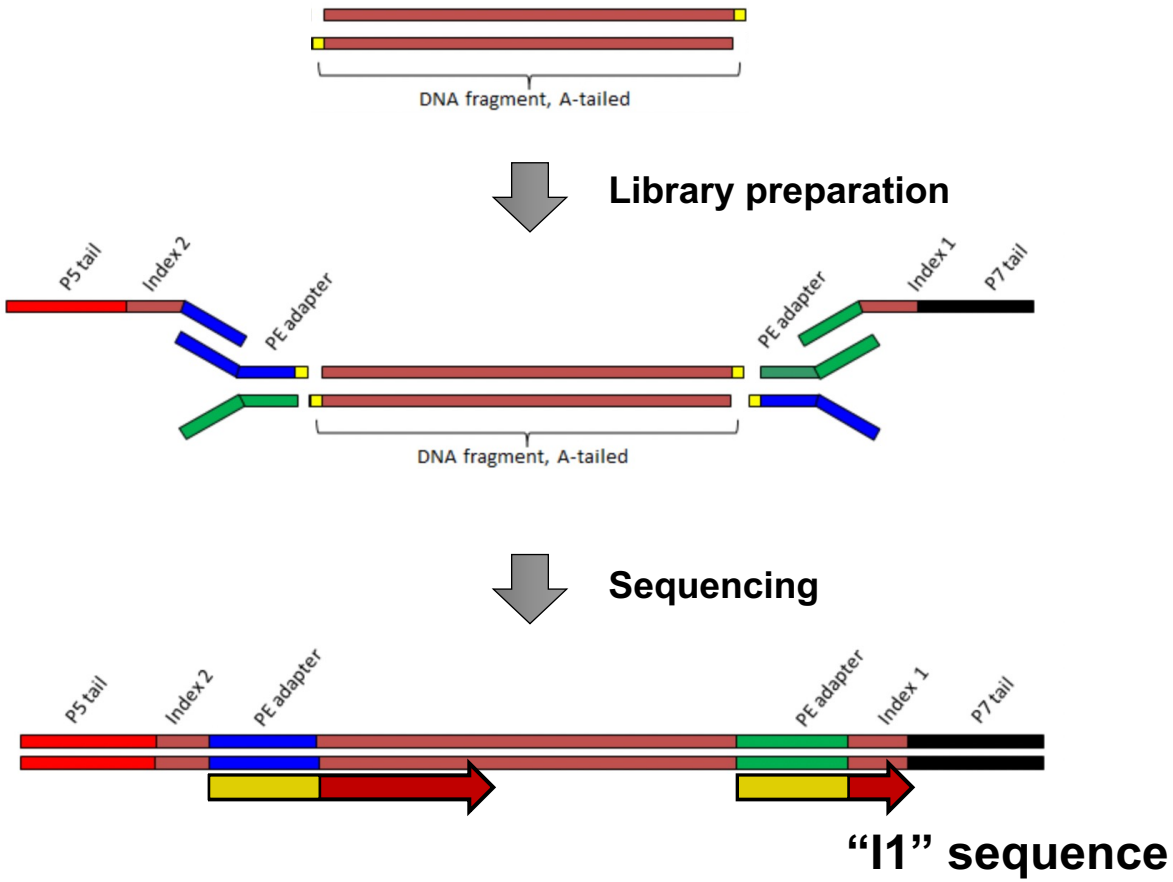

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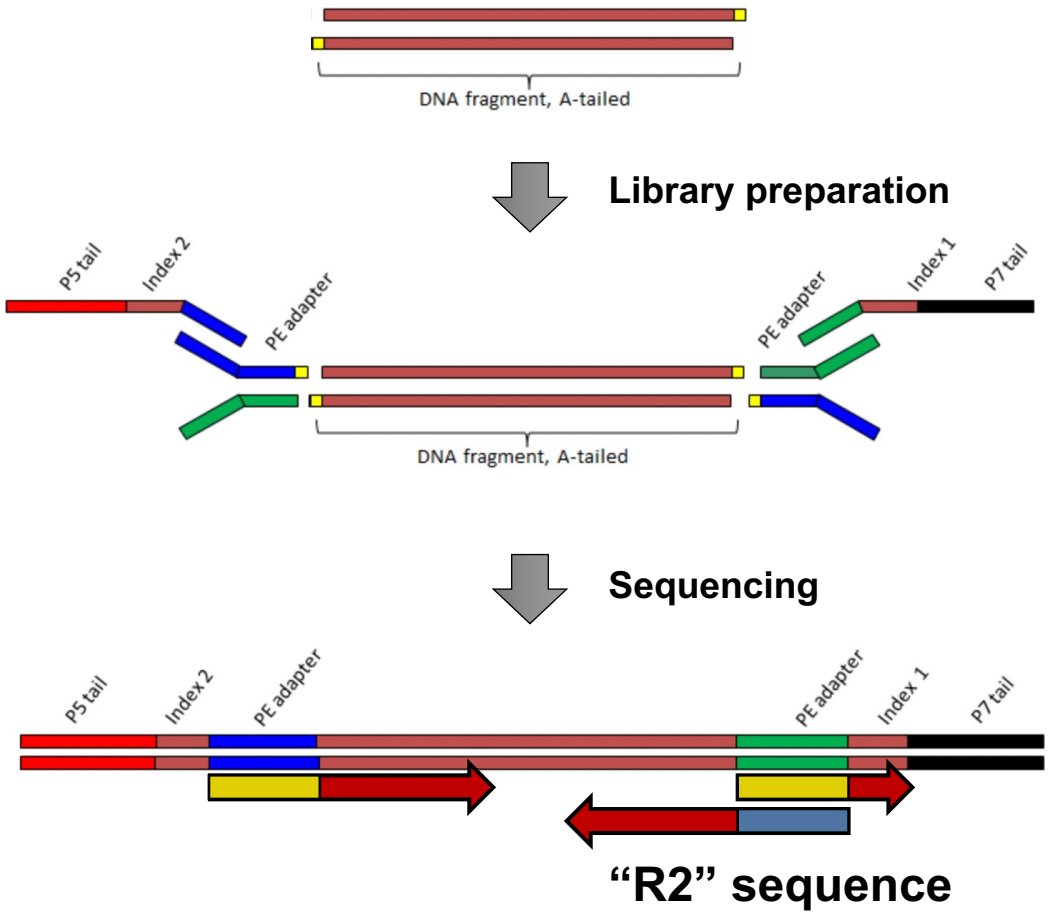

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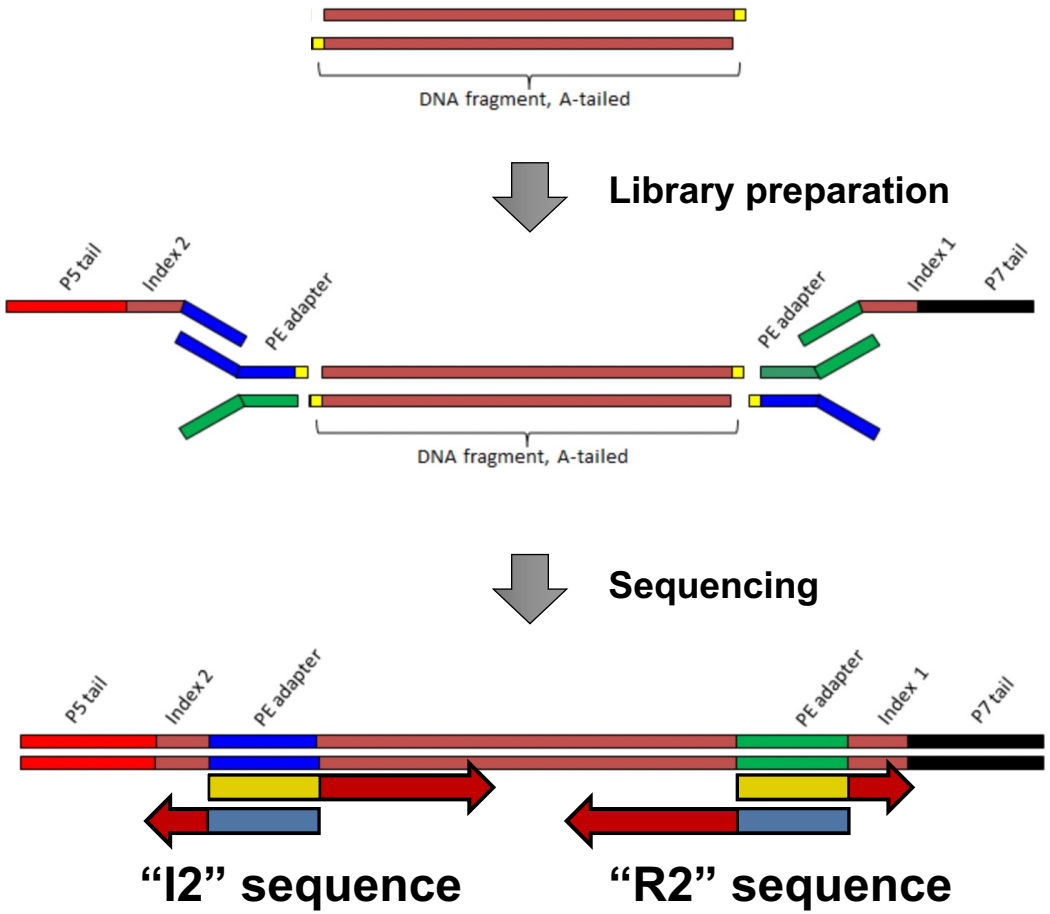

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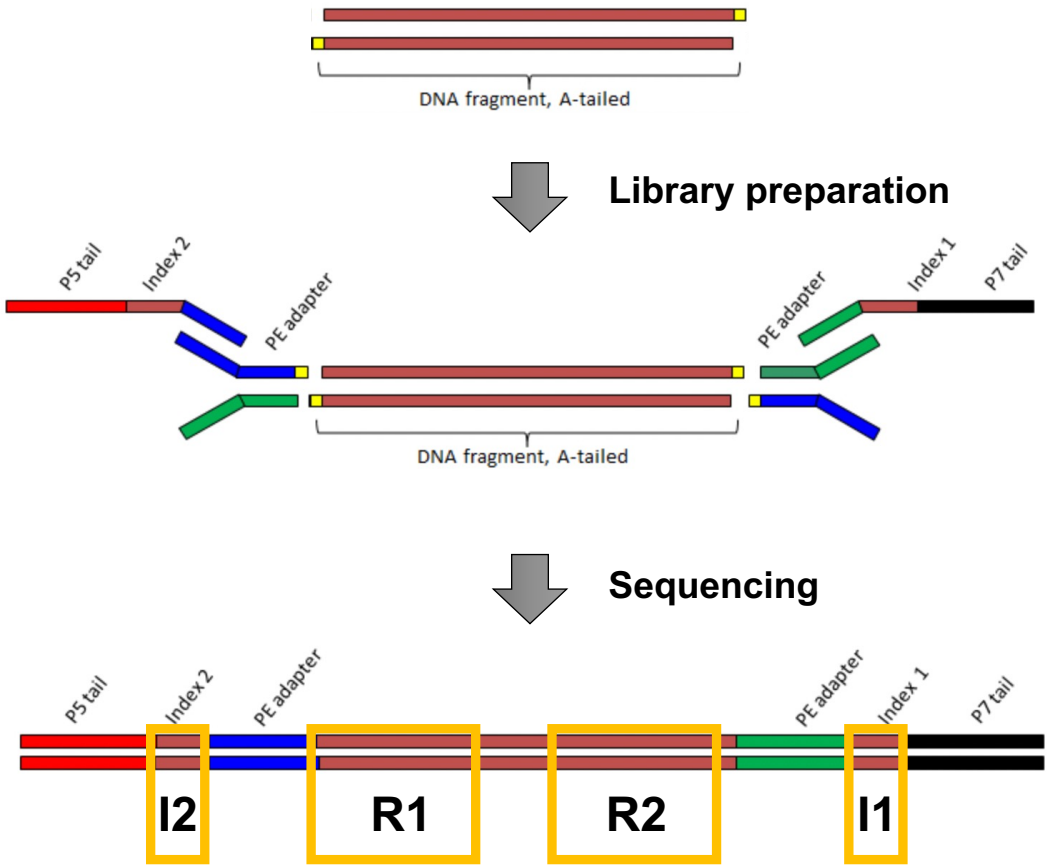

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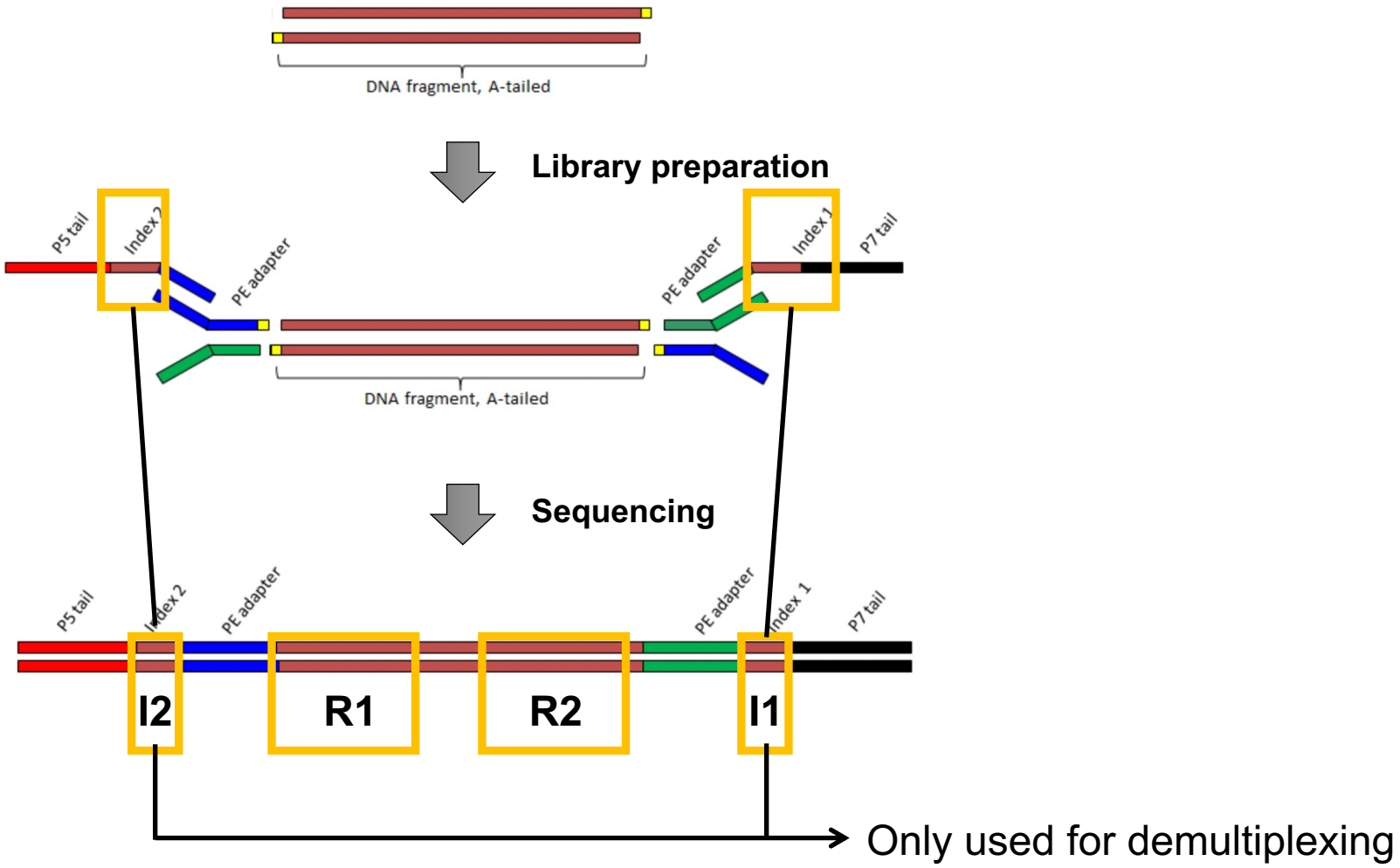

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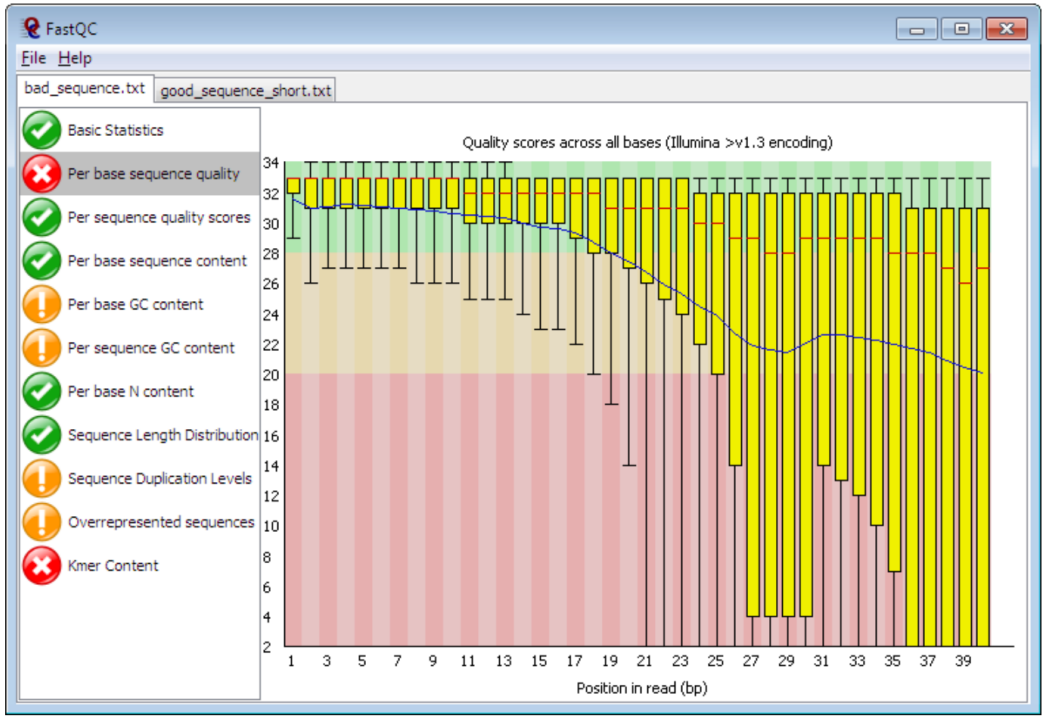
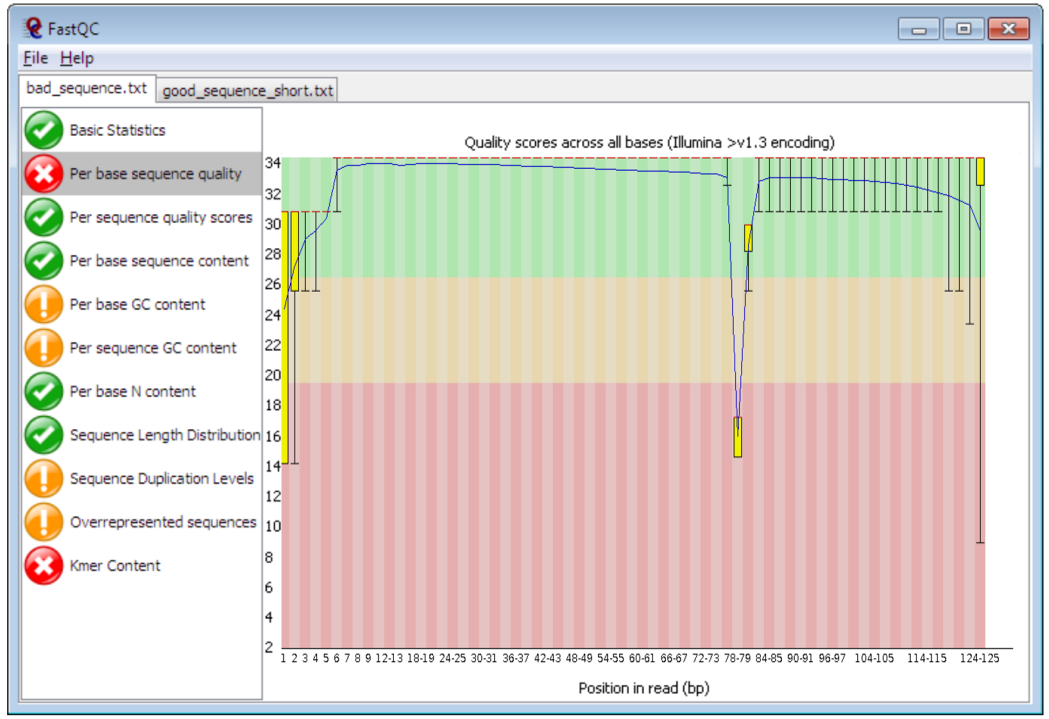
FastQC

FastQC is a program designed to spot potential problems in high througput sequencing datasets. It runs a set of analyses on one or more raw sequence files in fastq or bam format and produces a report which summarises the results.





FastQC will highlight any areas where this library looks unusual and where you should take a closer look. The program is not tied to any specific type of sequencing technique and can be used to look at libraries coming from a large number of different experiment types (Genomic Sequencing, ChIP-Seq, RNA-Seq, BS-Seq etc etc).

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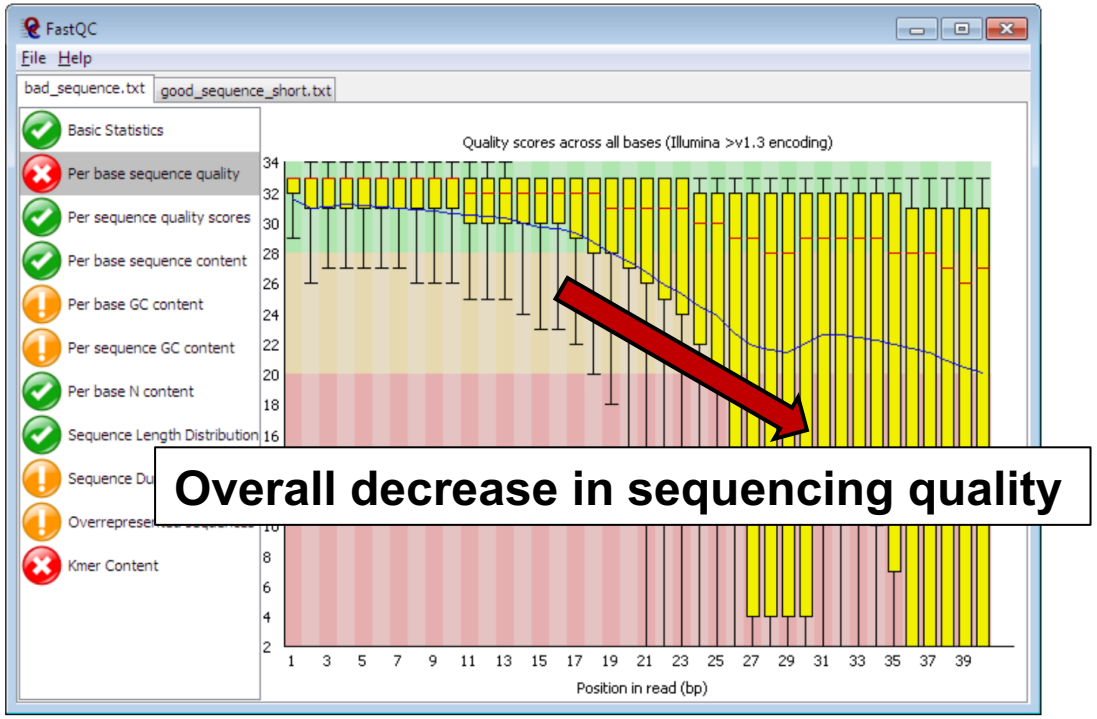

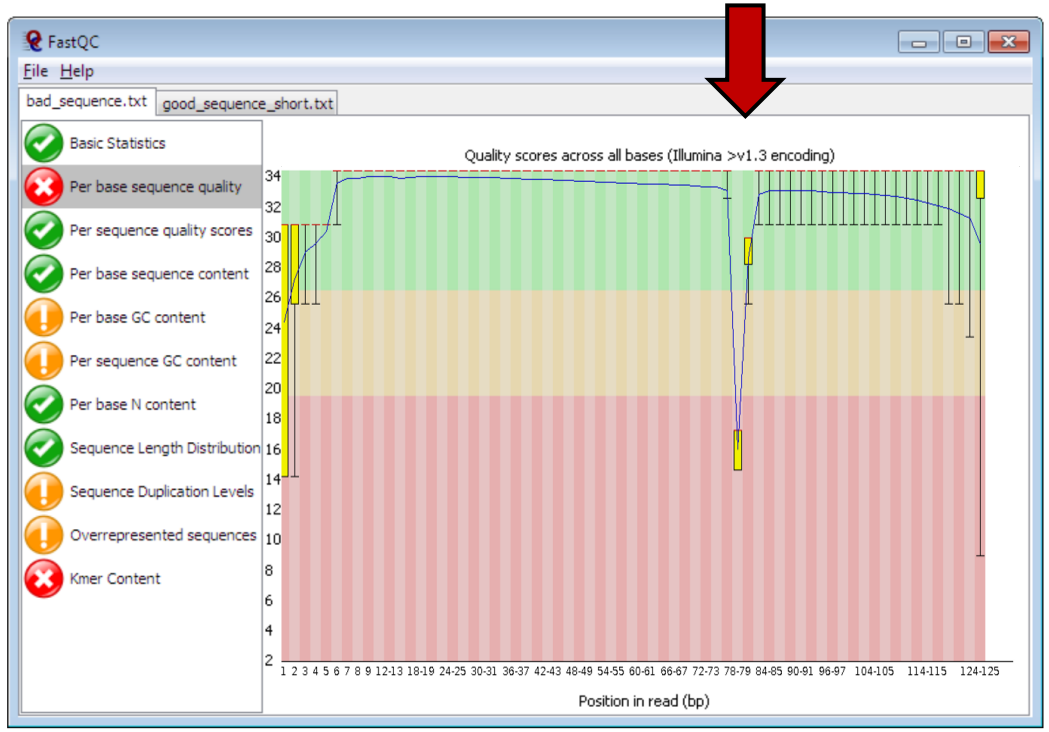

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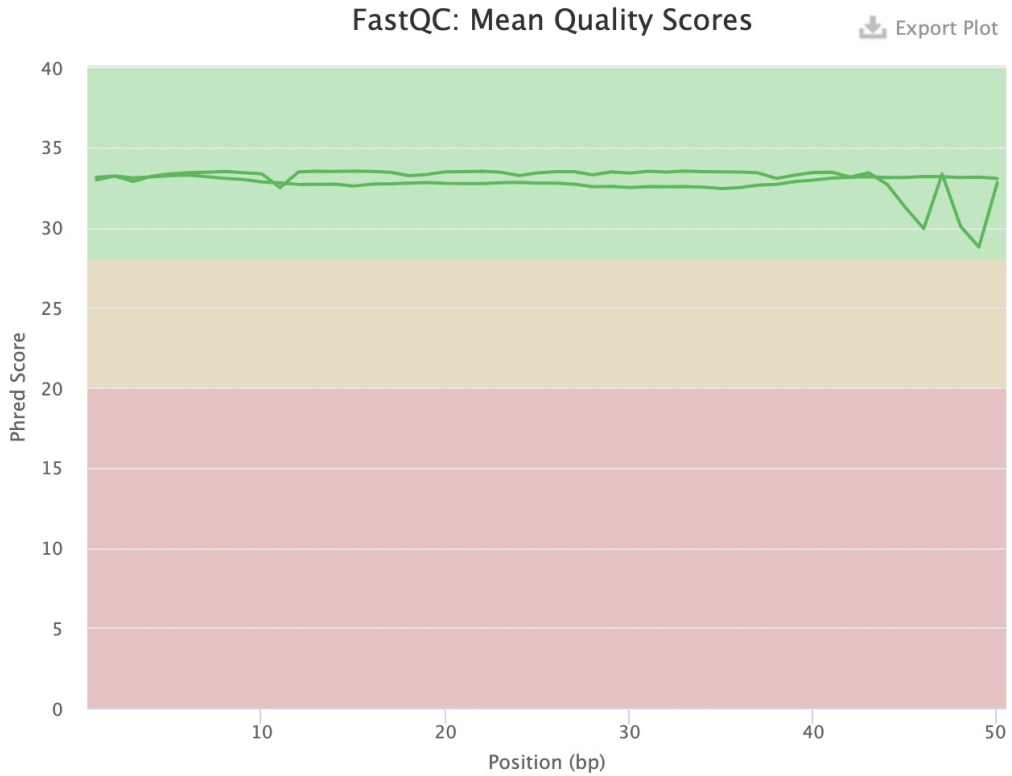
Cutadapt: trim away adapter sequences and low-quality ends



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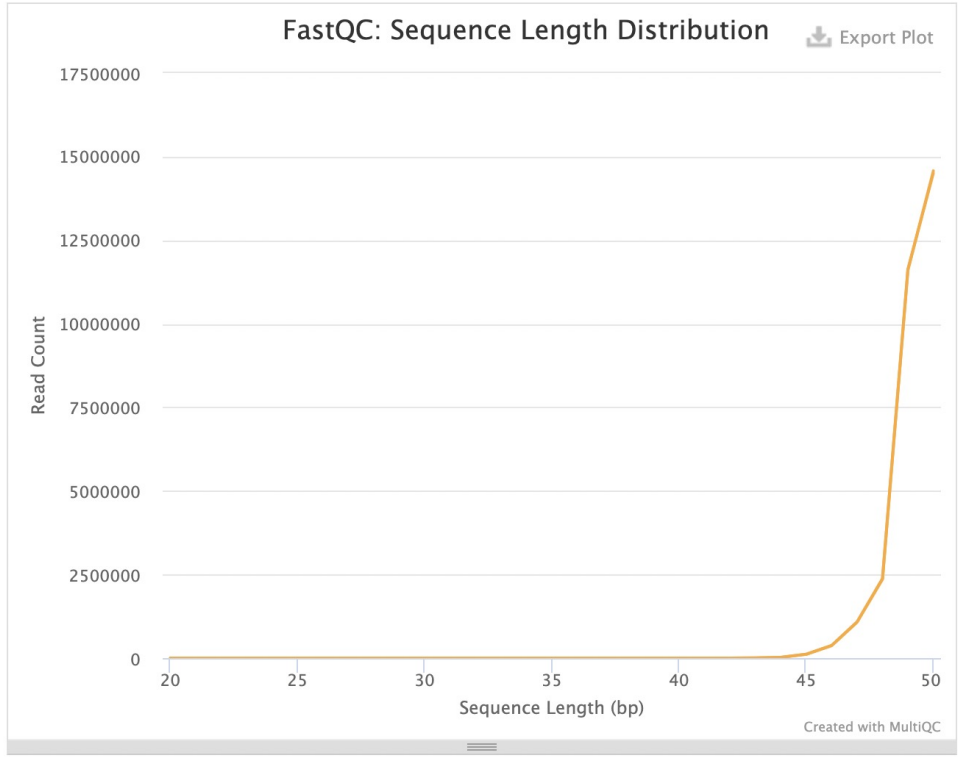
Sequence Length Distribution 80

All samples have sequences of a single length (50bp).



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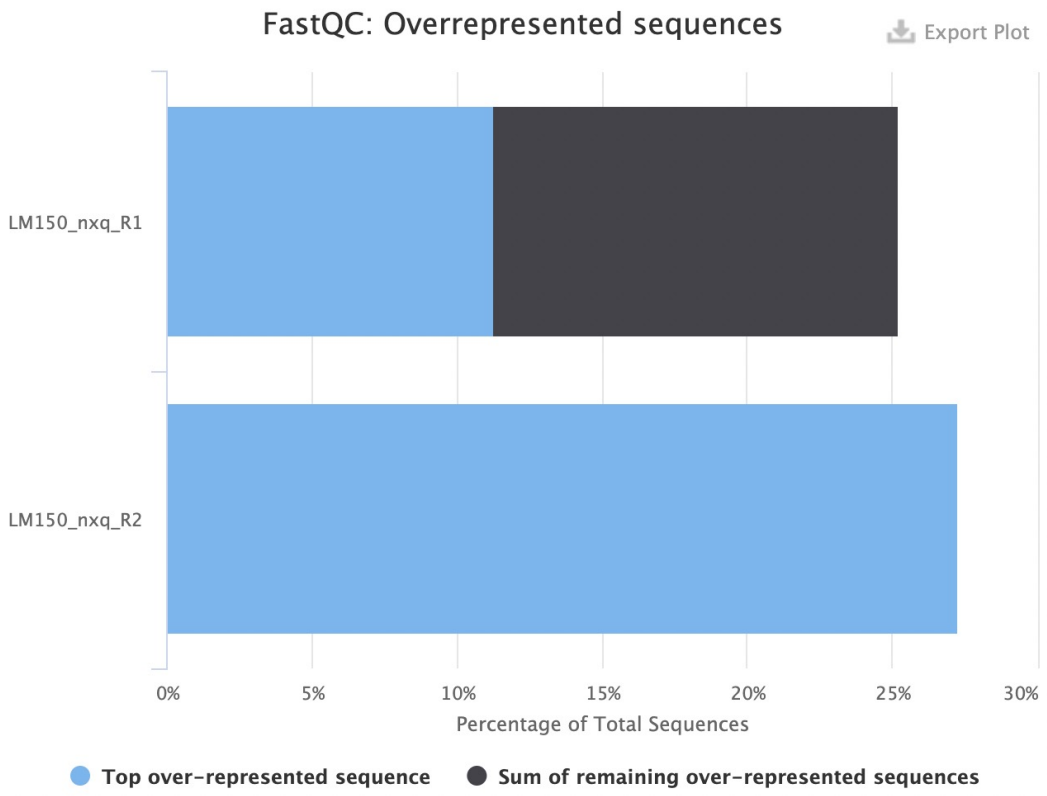

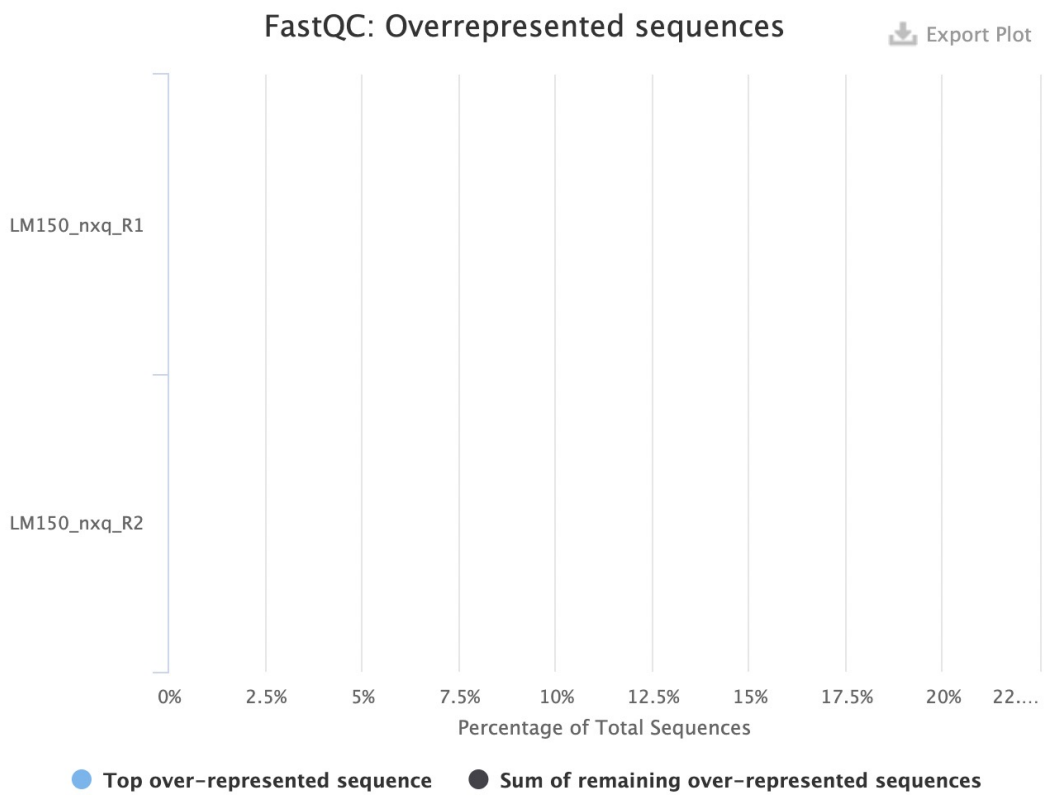

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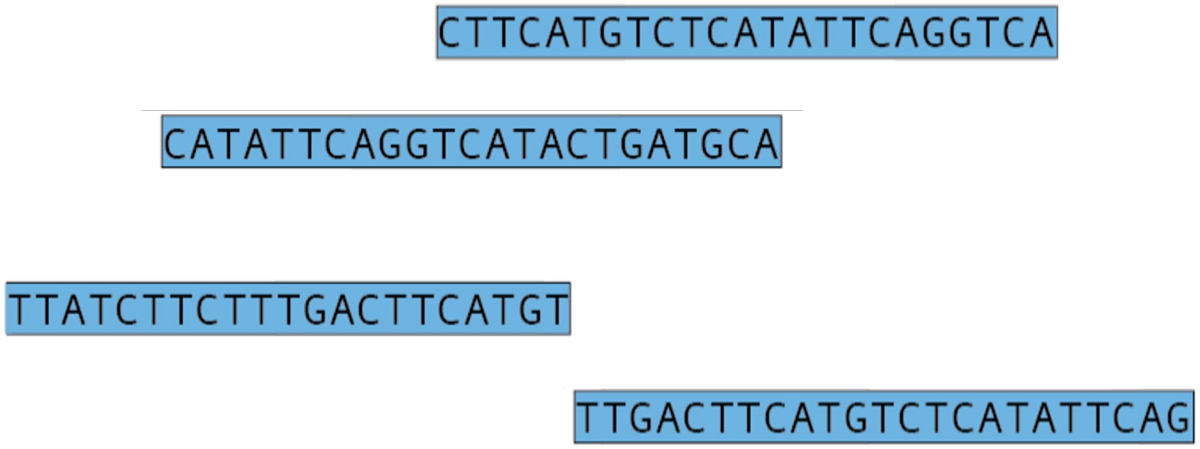
Mapping sequencing reads to a reference



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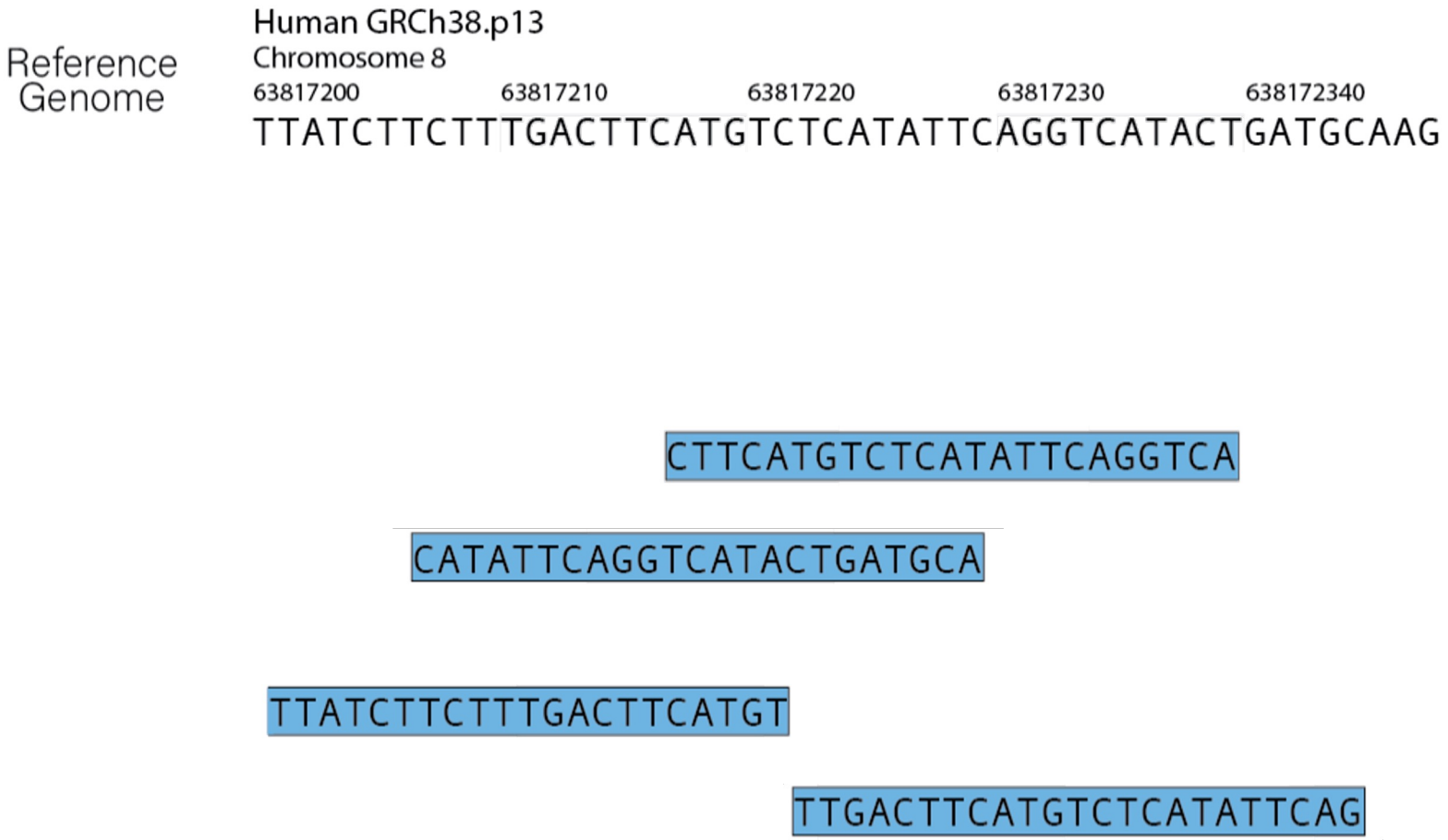

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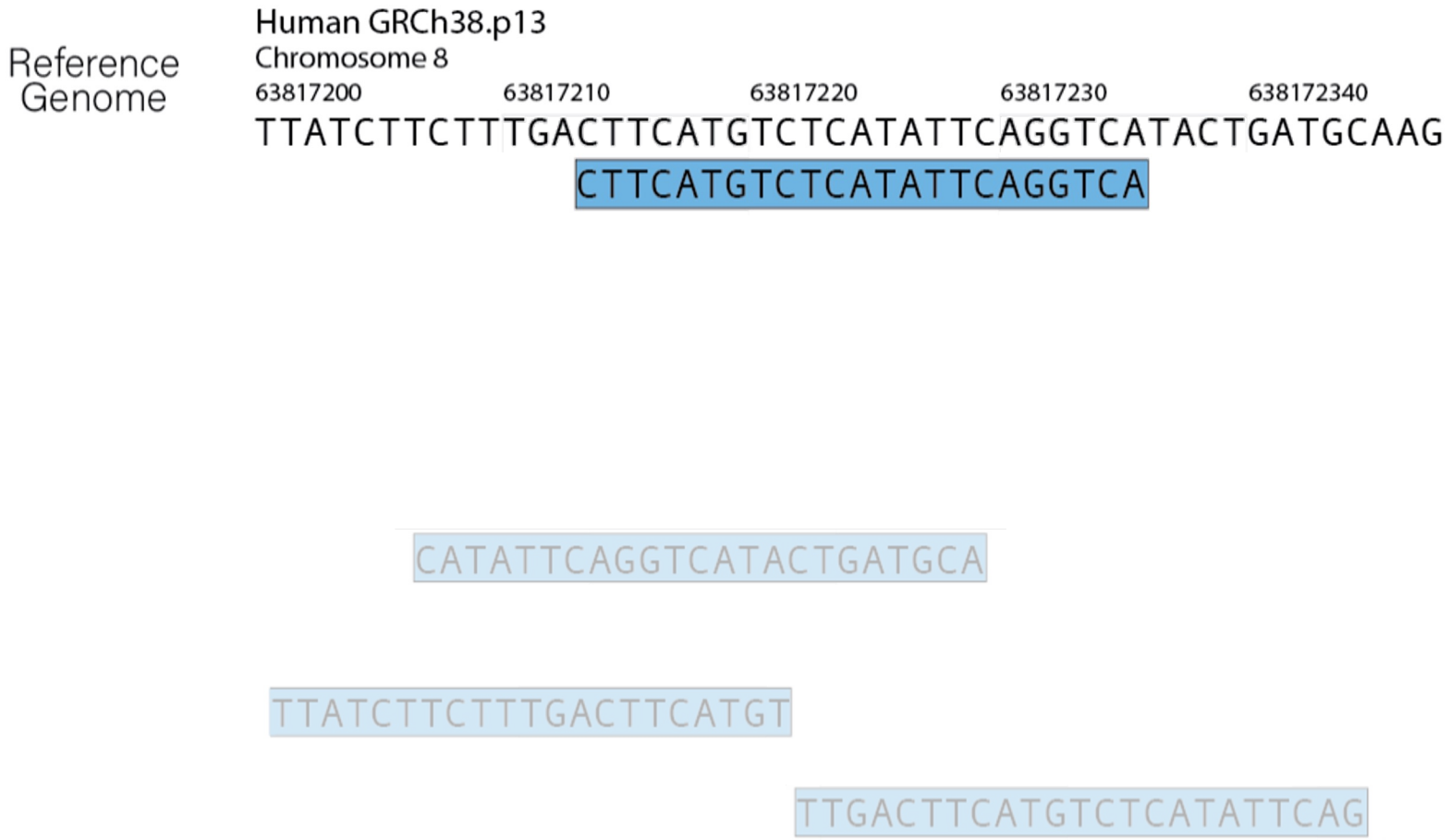

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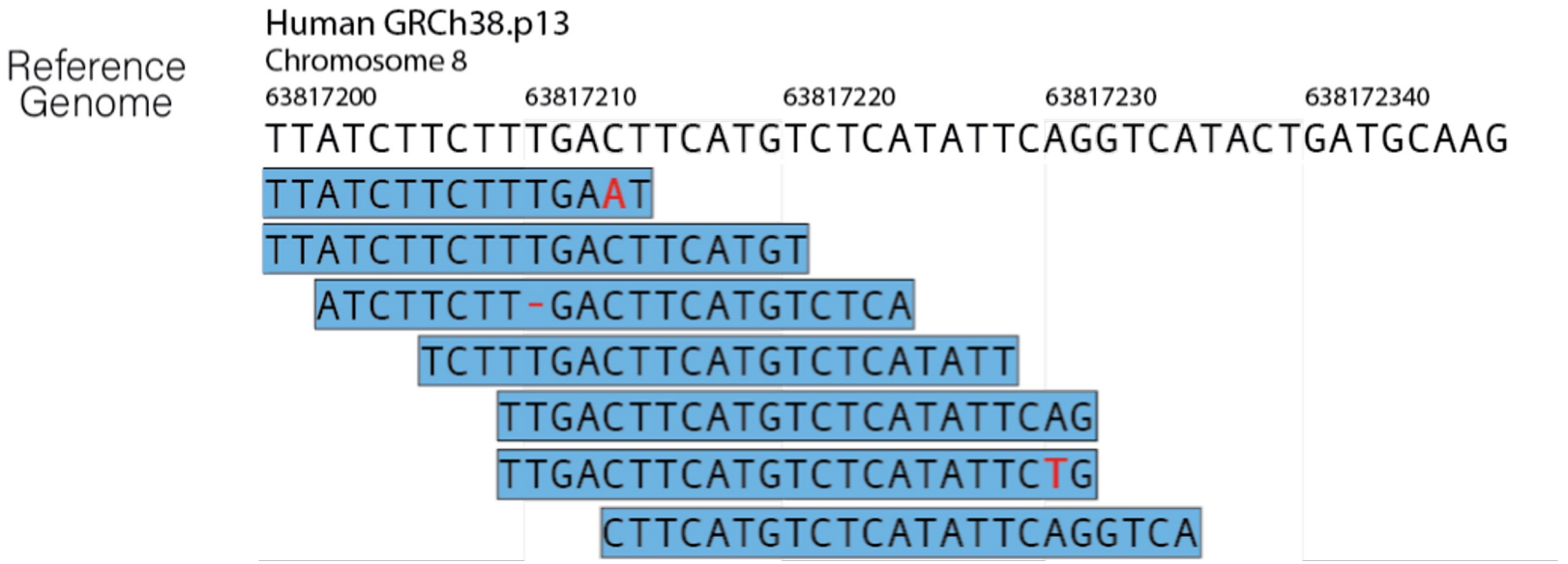

SAM file format

Sequence Alignment Map (SAM) is a human-readable, rectangular, text-based format for storing biological sequences aligned to a reference sequence.

Each entry (line) describes where a read is mapped on the reference and how it is mapped

Col	Field	Type	Brief description
1	QNAME	String	Query template NAME
2	FLAG	Int	bitwise FLAG
3	RNAME	String	References sequence NAME
4	POS	Int	1- based leftmost mapping POSition
5	MAPQ	Int	MAPping Quality
6	CIGAR	String	CIGAR string
7	RNEXT	String	Ref. name of the mate/next read
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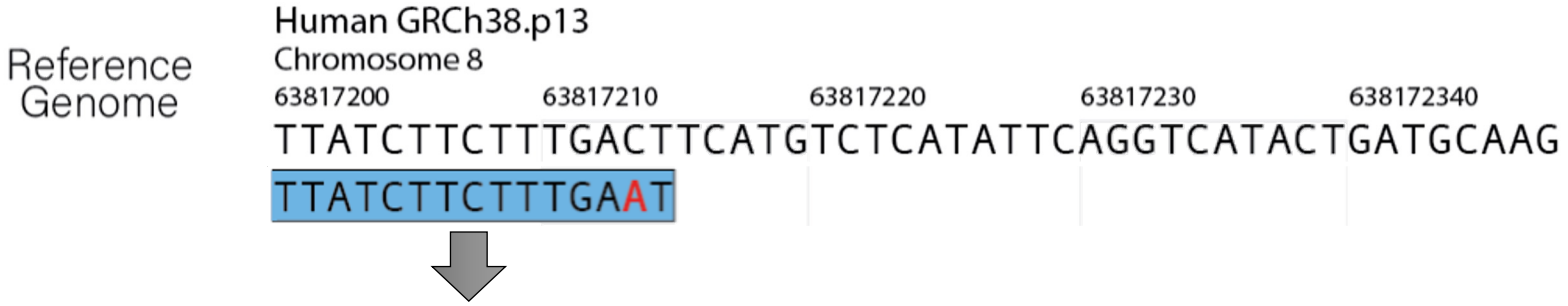


Read name	Flag	Chr	Position	Length	CIGAR	Read name (mate)	Chr (mate)	Position (mate)	Sequence	Per base sequencing quality
HWI-ST330:304:H045HADXX:2093#1	2	chr8	63817200	50	14M1X	2093#2	chr8	6381932	TTATCTTCTTTGAAT	?????BBBBBBDD=?

BAM file format

BAM files are **binarized** SAM files, allowing great compression of the alignment results.

However, bam files are not directly human-readable.

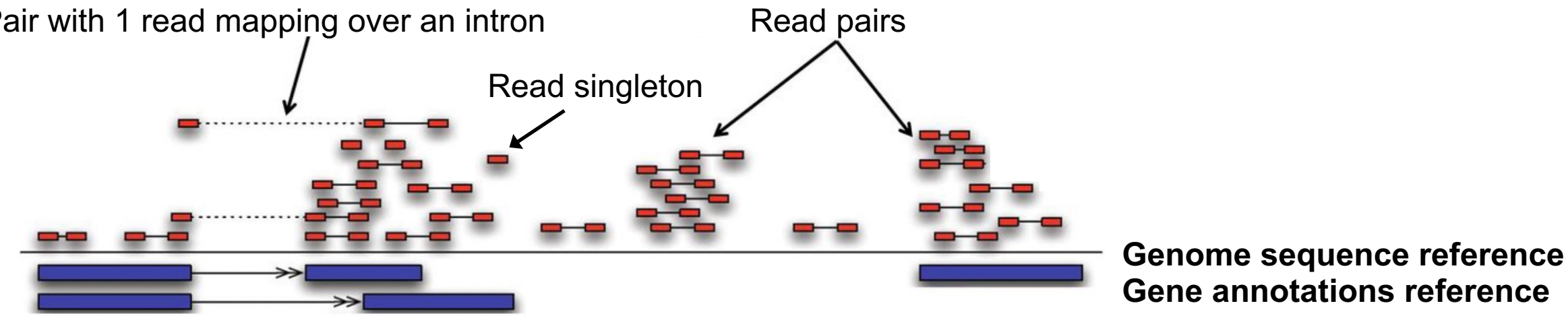
```
samtools view --bam ….sam > ….bam
```

Mapping tools

There are a plethora of alignment tools.

Each one requires the genome reference to be indexed first.

Some mappers can be ”**splice-aware**”, allowing reads to be mapped over annotated introns.



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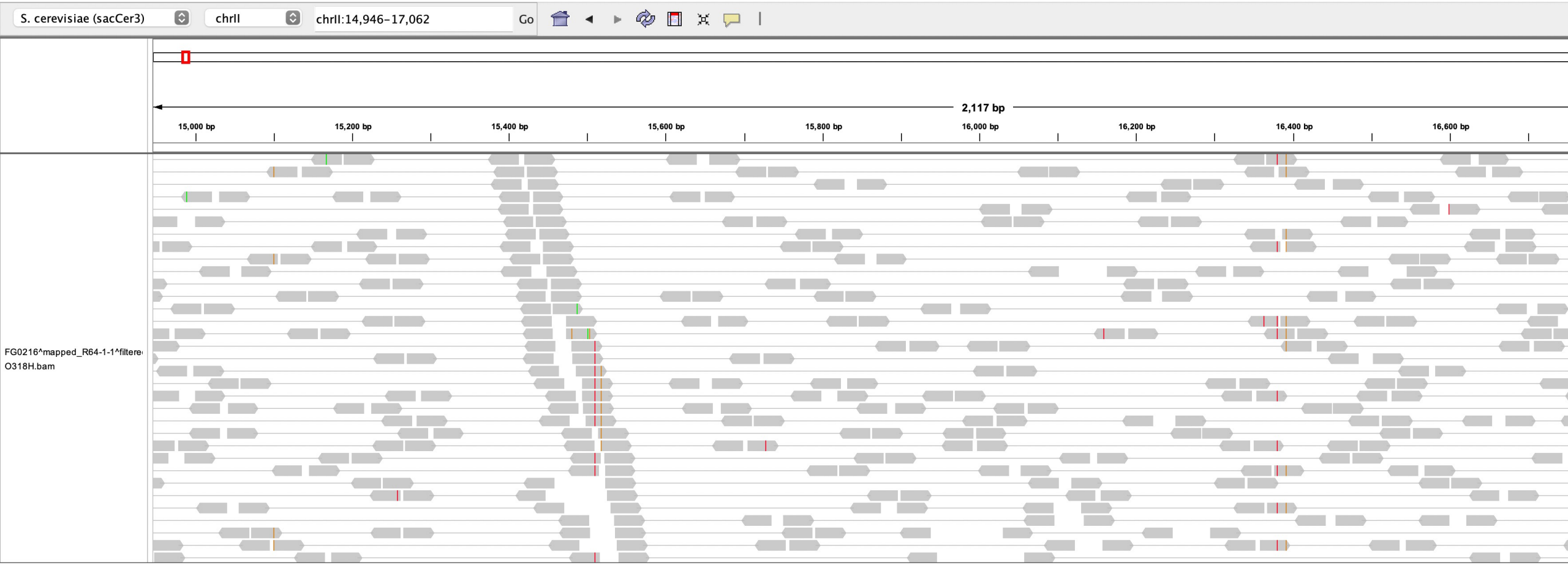
IGV: Integrative Genome Browser



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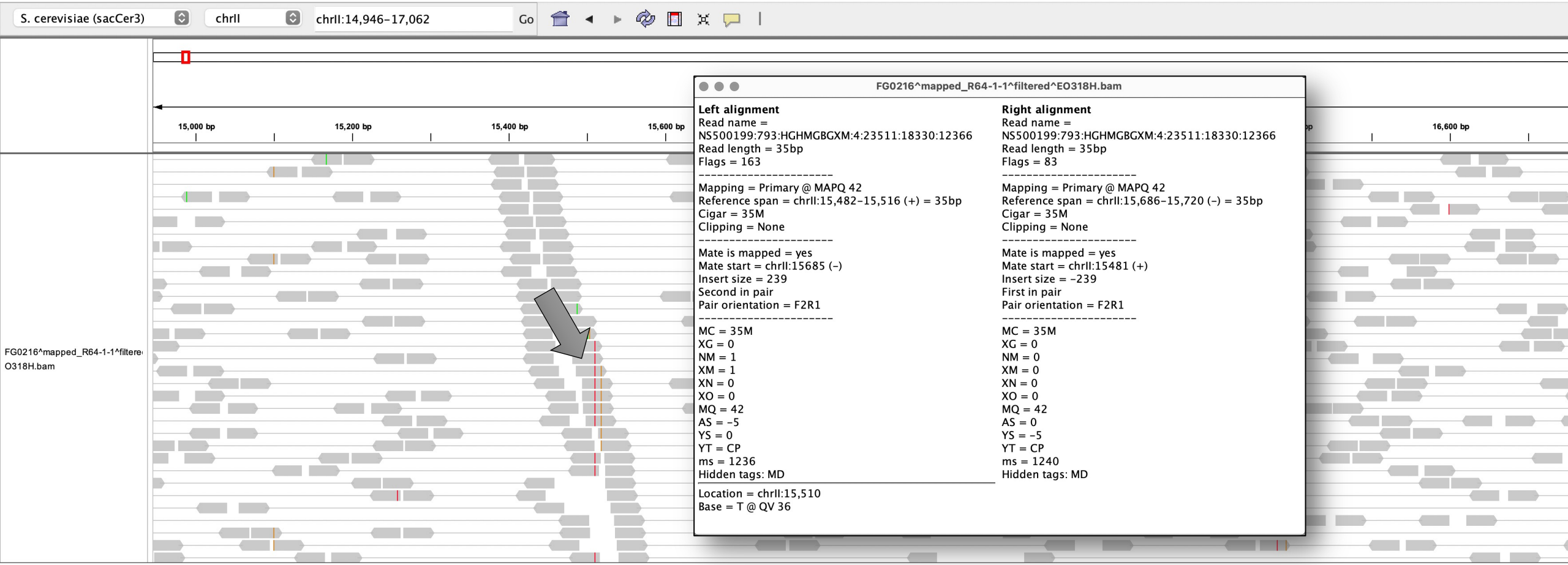

Filtering duplicates

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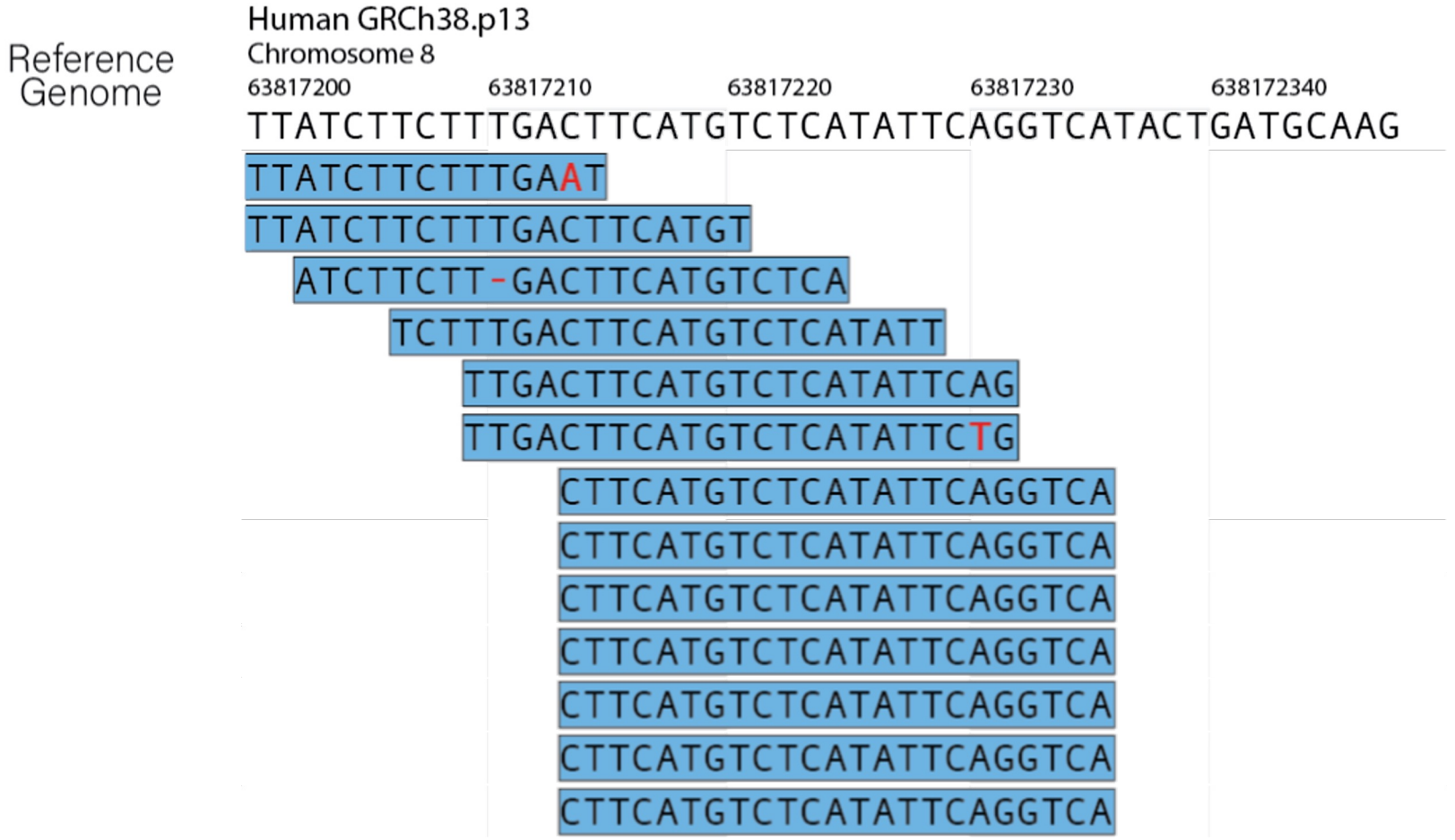

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Filtering out low-quality mapping reads

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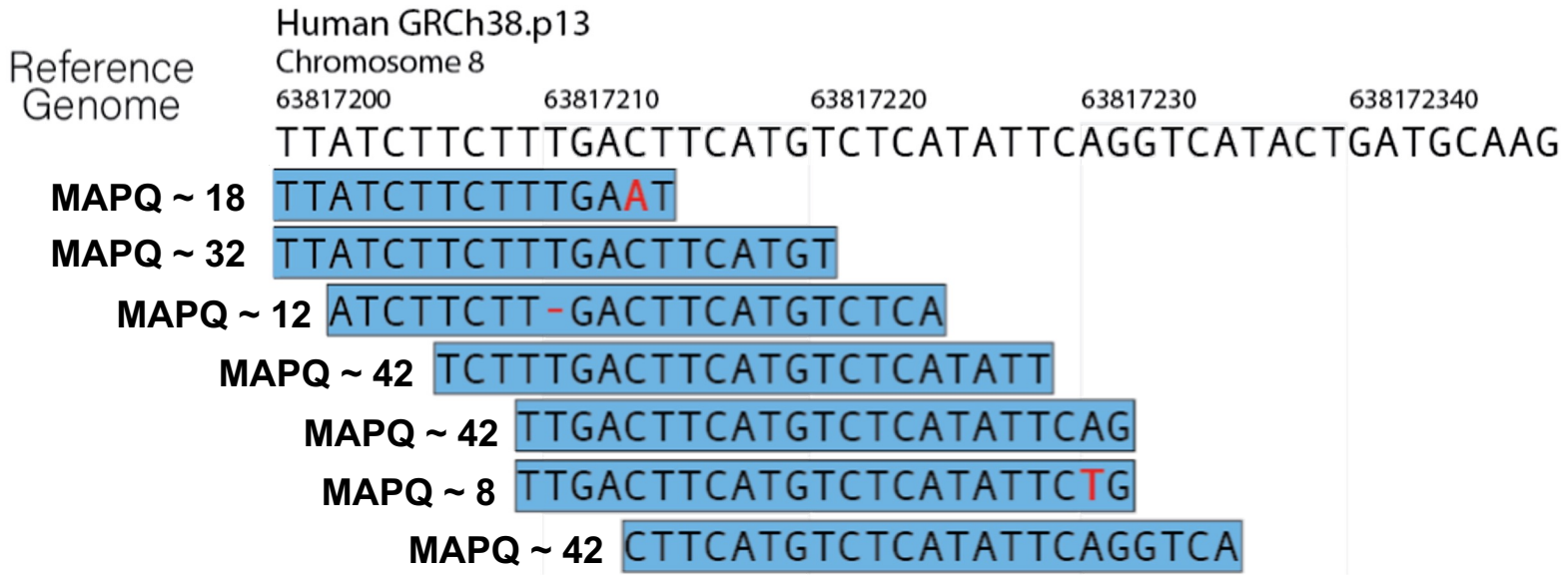
$$MAPQ = -10 * log_{10}\big(P(read\ is\ wrongly\ mapped)\big)$$



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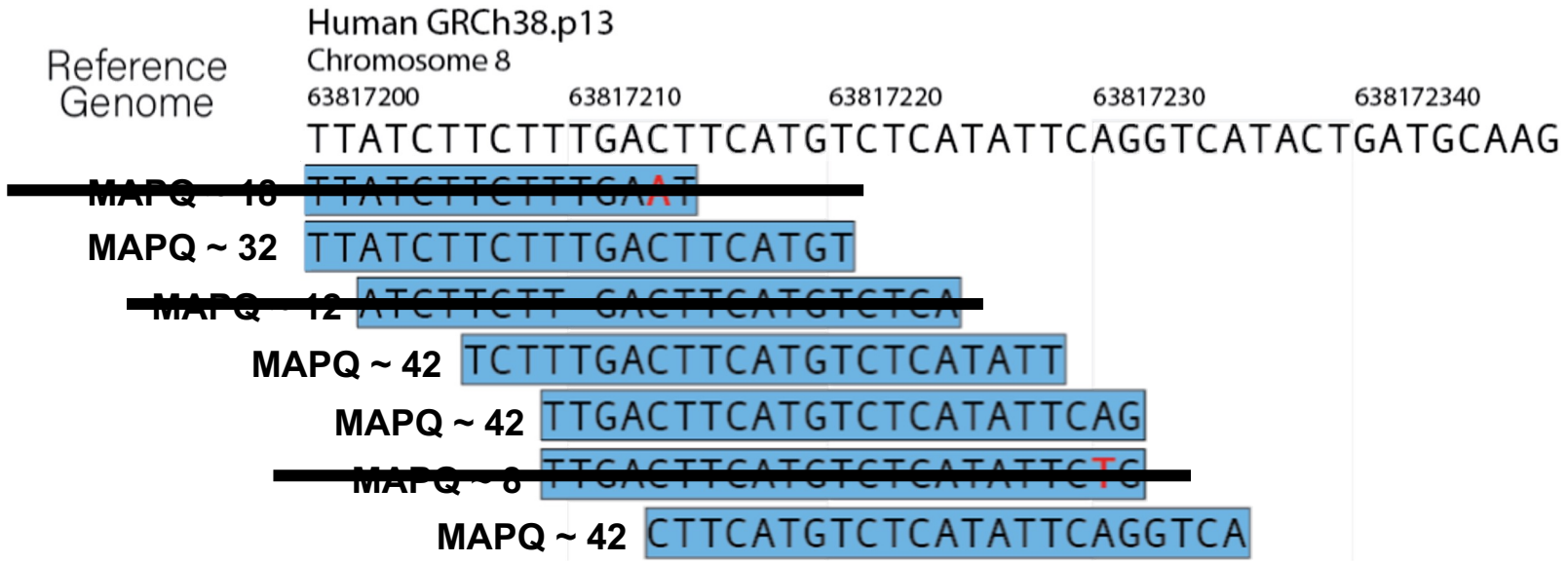
For example, a MAPQ score of 20 indicates that the probability for the read to be map at the indicated position is 0.01.



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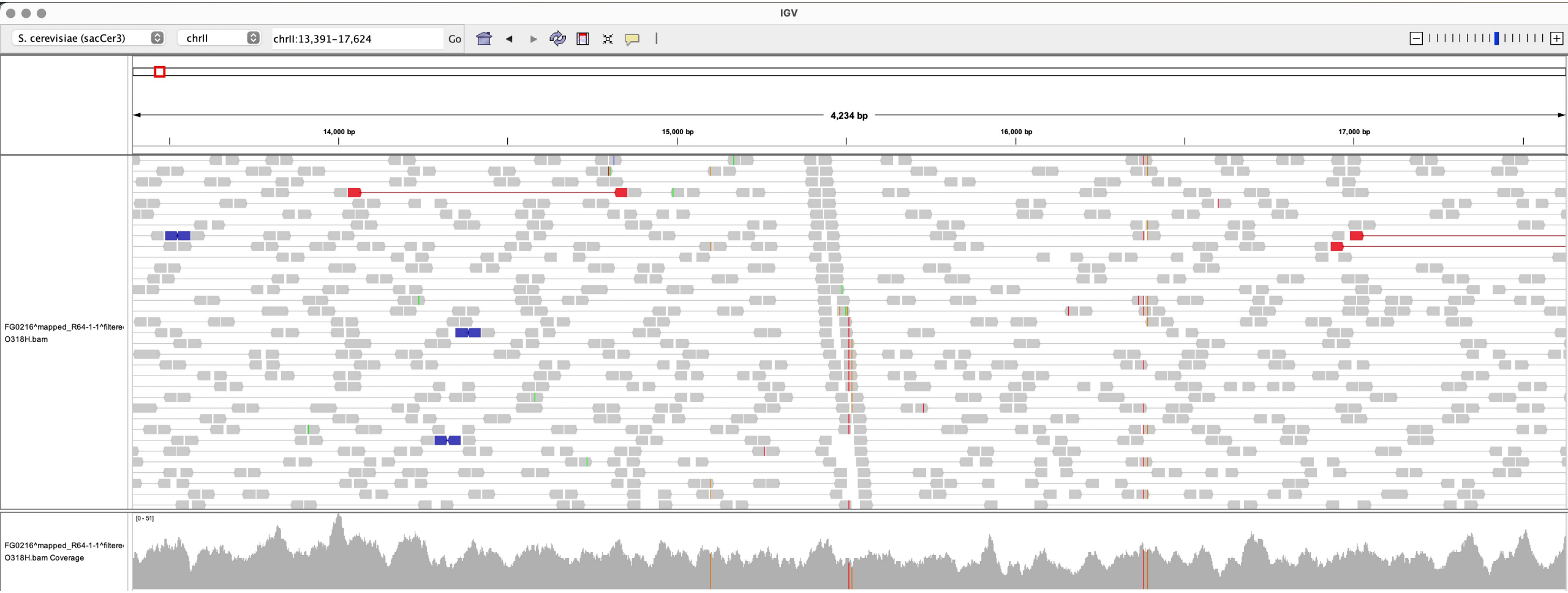
Generating tracks from mapped reads

Basic approach: ”pile-up” of all the fragments in `bam` files to generate a **coverage track**.



deepTools: a software suite to manage/produce genomic tracks

https://deeptools.readthedocs.io/en/latest/content/list_of_tools.html

- Tools for BAM and bigWig file processing
  - multiBamSummary
  - multiBigwigSummary
  - correctGCBias
  - bamCoverage
  - bamCompare
  - bigwigCompare
  - bigwigAverage
  - computeMatrix
  - alignmentSieve

- Tools for QC
  - plotCorrelation
  - plotPCA
  - plotFingerprint
  - bamPEFragmentSize
  - computeGCBias
  - plotCoverage
- Heatmaps and summary plots
  - plotHeatmap
  - plotProfile
  - plotEnrichment
- Miscellaneous
  - computeMatrixOperations
  - estimateReadFiltering

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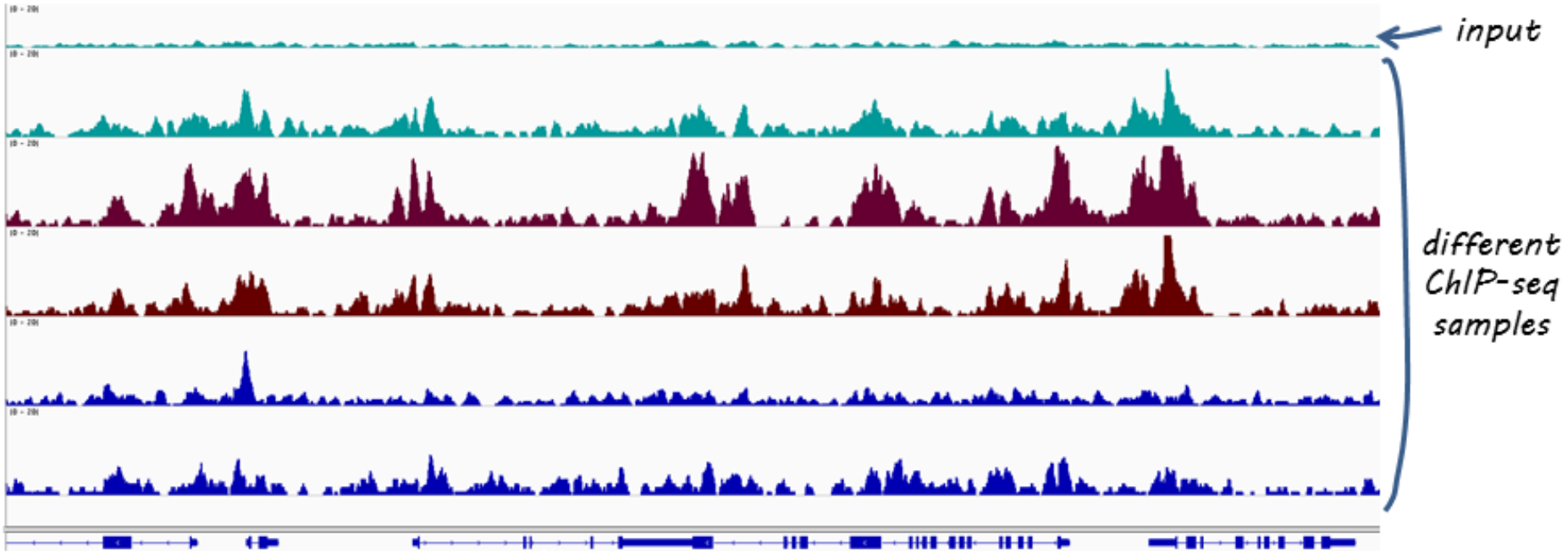
for visualizing continuous data, e·g· in the UCSC Genome Browser or IGV, bigWig files come in really handy



remember that there are 2 deepTools for bam → bigWig conversion:

- ***bamCoverage:*** *for individual files (like those shown here)*
- ***bamCompare:*** *to normalize two files to each other*

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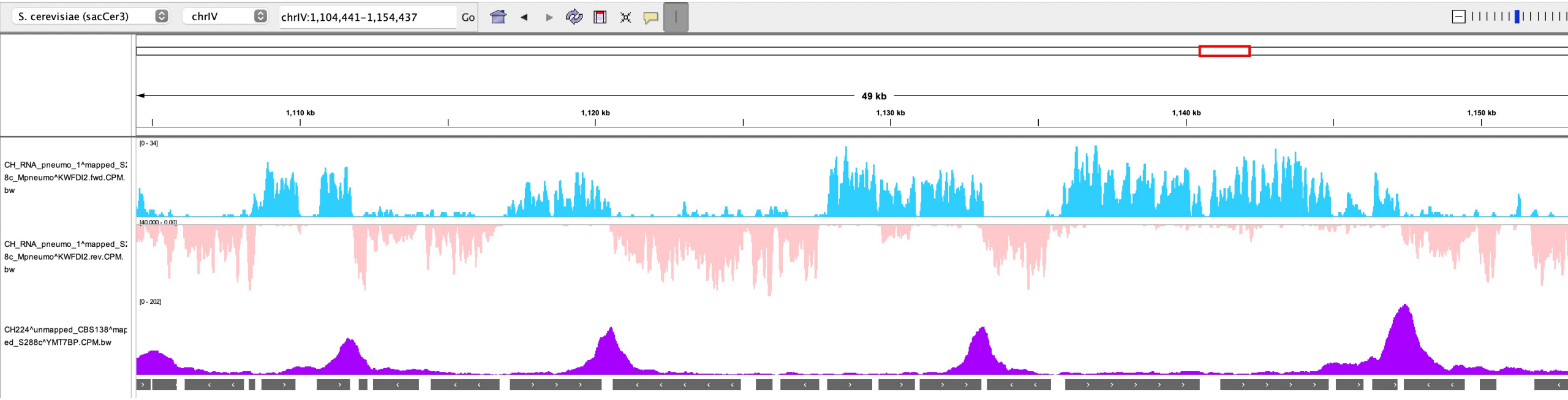

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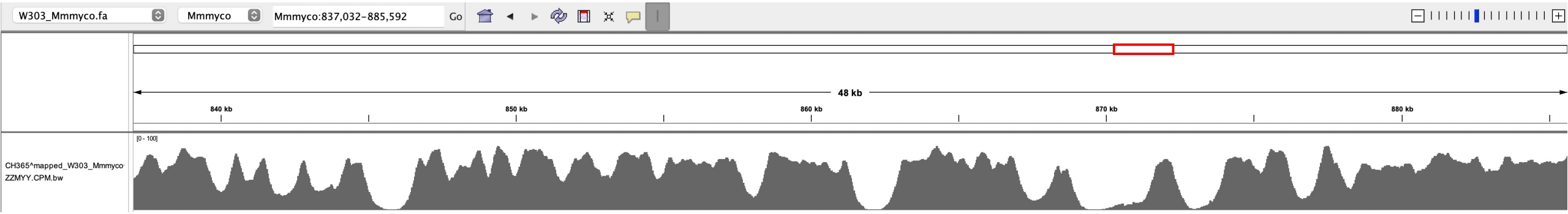

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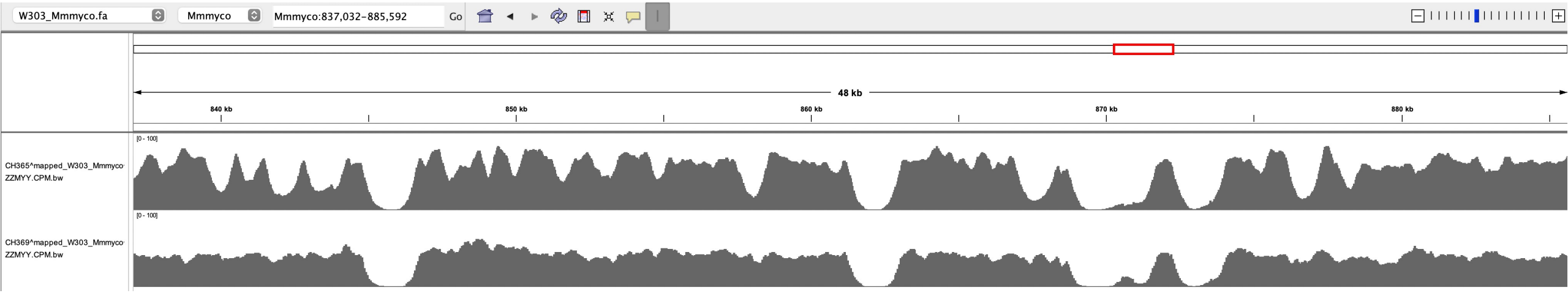

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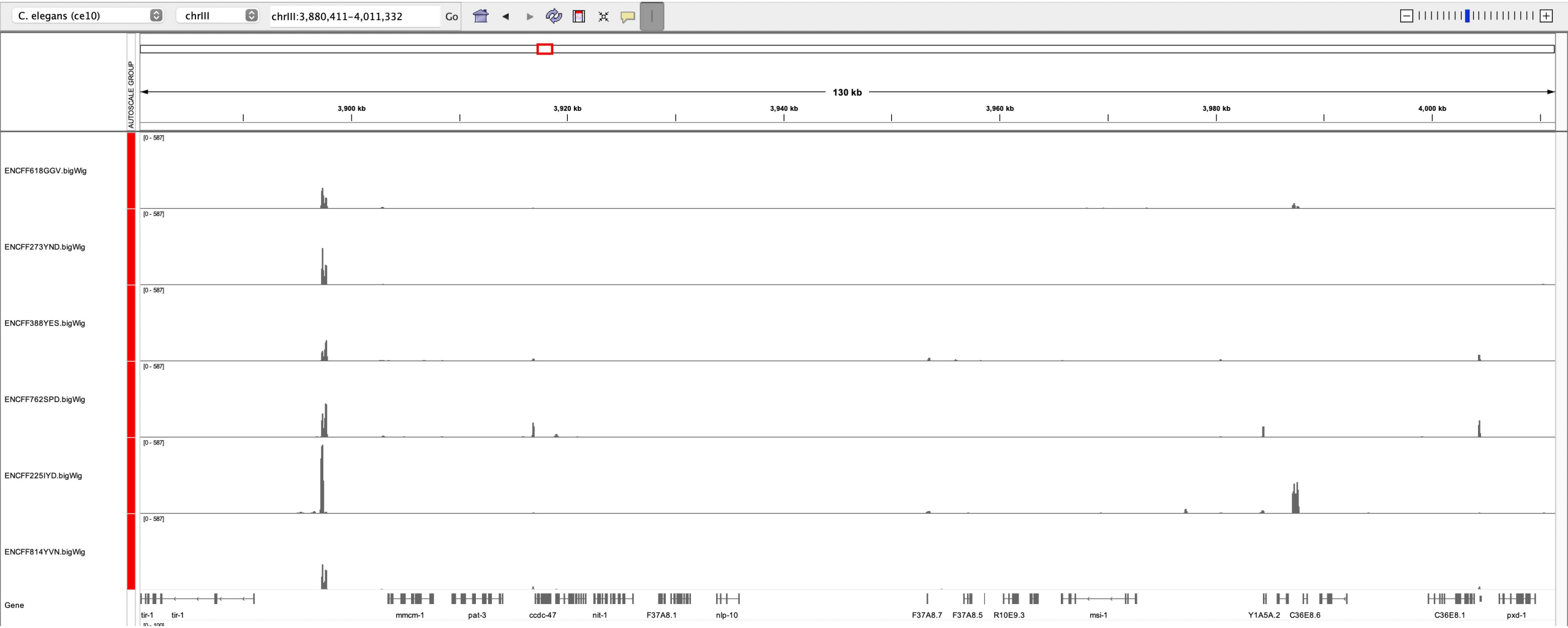

Watch out for artefacts

Genome-wide identification and characterisation of HOT regions in the human genome

Hao Li, Feng Liu, Chao Ren, Xiaochen Bo & Wenjie Shu

BMC Genomics **17**, Article number: 733 (2016) | Cite this article

Regulatory analysis of the *C. elegans* genome with spatiotemporal resolution

Carlos L. Araya, Trupti Kawli, Anshul Kundaje, Lixia Jiang, Beijing Wu, Dionne Vafeados, Robert Terrell, Peter Weissdepp, Louis Gevirtzman, Daniel Mace, Wei Niu, Alan P. Boyle, Dan Xie, Lijia Ma, John I. Murray, Valerie Reinke, Robert H. Waterston & Michael Snyder

Nature **512**, 400–405 (2014) | Cite this article

HOT regions are dynamic in development

JOURNAL ARTICLE

HOT or not: examining the basis of high-occupancy target regions

Katarzyna Wreczycka, Vedran Franke, Bora Uyar, Ricardo Wurmus, Selman Bulut, Baris Tursun, Altuna Akalin ✉ Author Notes

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Extreme HOT regions are CpG-dense promoters in *C. elegans* and humans

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