# Human Genomics and Epigenomics

Pratical 1 – 18/01/2021
**Pratical 2 – 19/01/2021**
Pratical 3 – 25/01/2021
Pratical 4 – 26/01/2021

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Functional Genomics lab

# ALIGNMENT AND VARIANT CALLING

**1° Day (3h): Pre-processing of raw reads**

- The fastq file
- Quality control of fastq files
- Adapter removing and trimming of fastq files
  - Sickle and scythe
  - Trimmomatic
- Reads alignment:
  - The human reference genome (hg19 and hg38, main differences)
  - The BAM file

**2° Day (3h): Alignment**

- Alignment of trimmed reads to the reference genome
  - BWA-mem
  - Isaac2 pipeline
- Duplicates removal
- Read Clipping
- Visualization of aligned reads on IGV

# ALIGNMENT AND VARIANT CALLING

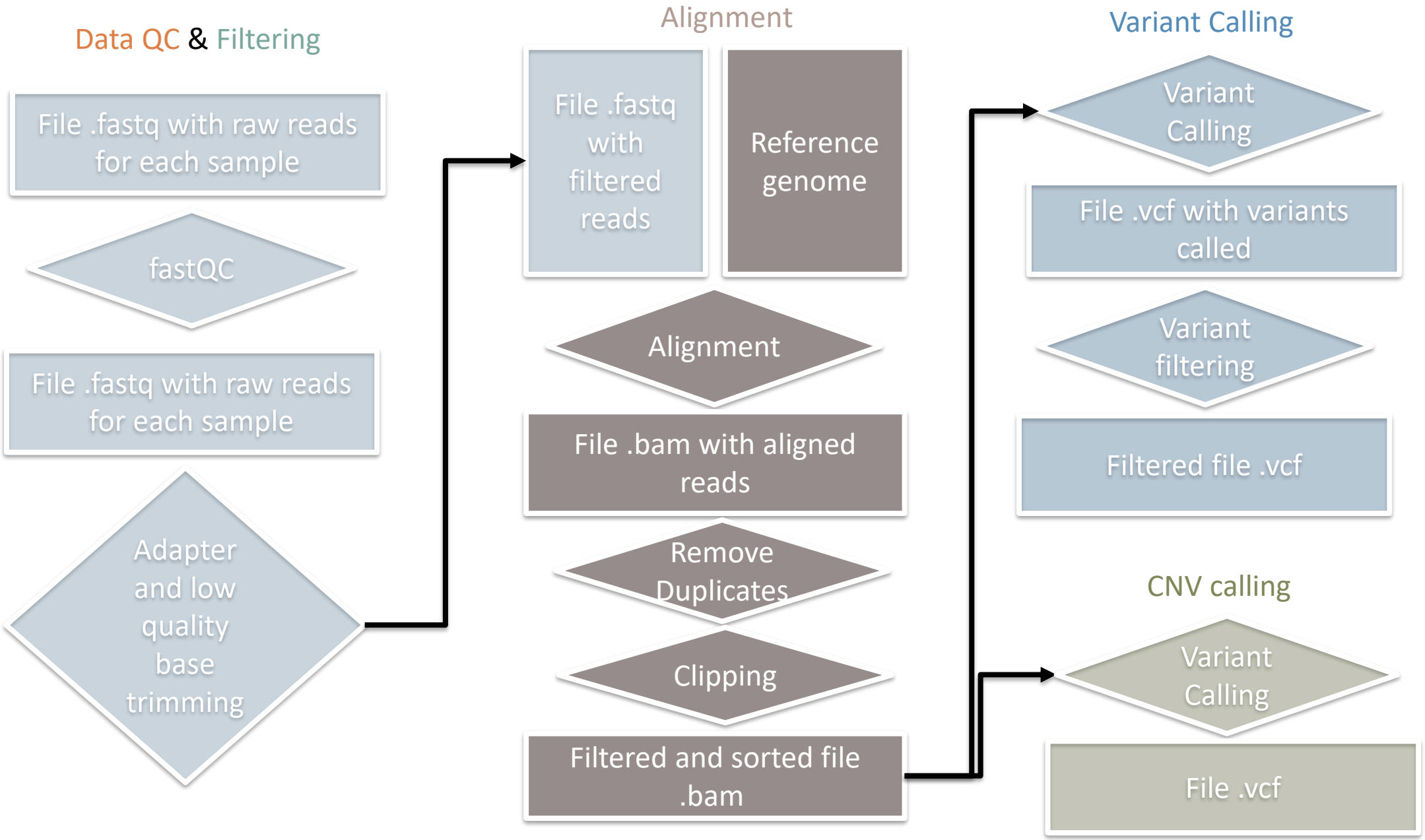
**3° Day (3h): Statistics and Variant Calling**

- Statistics on reads alignment: main parameters for the evaluation of NGS data
  - Average coverage and uniformity
  - Fold enrichment (on/near/off target)
  - Genotypability (mapping quality besides coverage)
- Variant calling:
  - The VCF and gVCF files
  - Germline variant calling
    - GATK4 Best practice pipeline

**4° Day (3h): Variant Calling**

- Germline variant calling
  - GATK4 Best practice pipeline
  - Strelka2
- Visualization of genetic variants on IGV
- CNV detection

# Pipeline


Data QC & Filtering
File .fastq with raw reads for each sample
fastQC
File .fastq with raw reads for each sample
Adapter and low quality base trimming
Alignment
File .fastq with filtered reads
Reference genome
Alignment
File .bam with aligned reads
Remove Duplicates
Clipping
Filtered and sorted file .bam
Variant Calling
Variant Calling
File .vcf with variants called
Variant filtering
Filtered file .vcf
CNV calling
Variant Calling
File .vcf

# ALIGNMENT OF TRIMMED READS TO THE REFERENCE GENOME

# BWA

- BWA is a software package for mapping low-divergent sequences against a large reference genome.

- Three different algorithms:
  - MEM
  - SW
  - backtrack

# BWA

- BWA is a software package for mapping low-divergent sequences against a large reference genome.

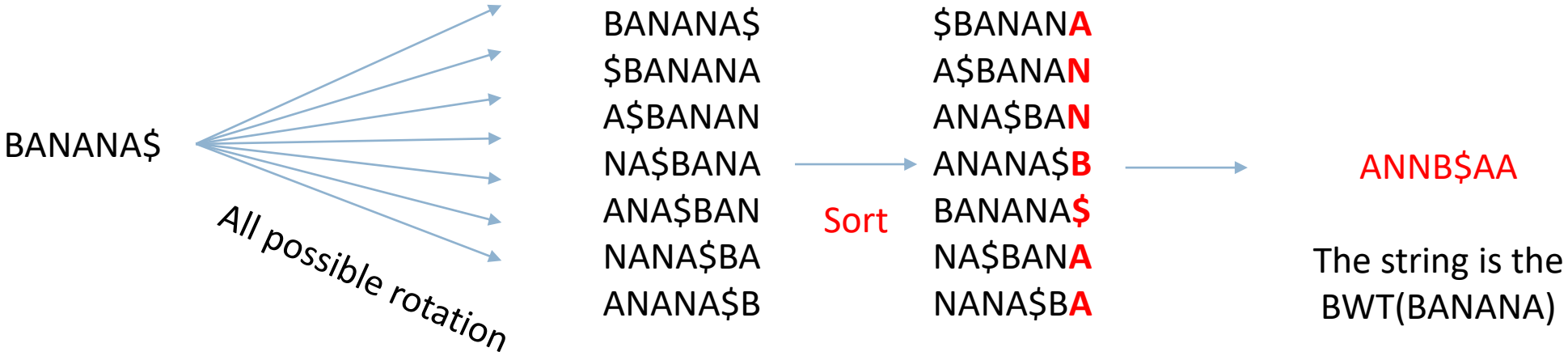
- Three different algorithms:
  - **MEM**
  - SW
  - backtrack

**Fast and accurate short read alignment with Burrows-Wheeler transform (2009)**
Heng Li, Richard Durbin
DOI: 10.1093/bioinformatics/btp324

# BWA-mem

**BWA-mem** is one of the most popular NGS aligners. Based on the Burrow Wheeler transform algorithm, BWA produces very accurate alignments. **It is used especially for DNA sequencing data: targeting sequencing, WES, WGS. It breaks reads up to 100bp. BWA soft-clip unmapped bases, retaining only reads with minimum 19bp mapped.**

## Burrow Wheeler Transform



# BWA-mem: Reconstruct «genome»

We have only the BWT and we want to reconstruct our reference sequence from the BWT(BANANA) = ANNB$AA

$BANANA
A$BANAN
ANA$BAN
ANANA$B
BANANA$
NA$BANA
NANA$BA

**IMPORTANT:** OUR BWT IS SORTED!

$BANANA
A$BANAN
ANA$BAN
ANANA$B
BANANA$
NA$BANA
NANA$BA

1. To reconstruct the first column sort the last one (BWT)

**IMPORTANT:** EACH ROW IS A CYCLYC ROTATION

A$
NA
NA
BA
$B
AN
AN

2. Now, we know 2-mer composition of the circular string

$B
AN
AN
A$
BA
NA
NA

3. Sort the 2-mer composition, gives us the first two columns of the matrix

# BWA-mem: Reconstruct «genome»

Now as before…

$BANANA
A$BANAN
ANA$BAN
ANANA$B
BANANA$
NA$BANA
NANA$BA

**IMPORTANT:**
EACH ROW IS A CYCLYC ROTATION

A$B
NA$
NAN
BAN
$BA
ANA
ANA

4. Now, we know 3-mer composition of the circular string

$BA
A$B
ANA
ANA
BAN
NA$
NAN

5. Sort the 3-mer composition, gives us the first three columns of the matrix

…and repeat again, till you reconstruct the full matrix

$BANANA
A$BANAN
ANA$BAN
ANANA$B
BANANA$
NA$BANA
NANA$BA

6. We assume that «$» is the lexicographically first, everything after must be the original sequence

# BWA-mem: Reconstruct «genome» faster

For each letter a number indicating the occurence is given.

We know that «$» is the starting point.

$1BANANA1
A1$BANAN1
A2NA$BAN2
A3NANA$B1
B1ANANA$1
N1A$BANA2
N2ANA$BA3

So we look at the character in the last column.

A$

$1BANANA1
A1$BANAN1
A2NA$BAN2
A3NANA$B1
B1ANANA$1
N1A$BANA2
N2ANA$BA3

In this case the occurence of the A is 3, so we go to the corresponding letter in the first column

NA$

$1BANANA1
A1$BANAN1
A2NA$BAN2
A3NANA$B1
B1ANANA$1
N1A$BANA2
N2ANA$BA3

ANA$

$1BANANA1
A1$BANAN1
A2NA$BAN2
A3NANA$B1
B1ANANA$1
N1A$BANA2
N2ANA$BA3

NANA$

$1BANANA1
A1$BANAN1
A2NA$BAN2
A3NANA$B1
B1ANANA$1
N1A$BANA2
N2ANA$BA3

ANANA$

$1BANANA1
A1$BANAN1
A2NA$BAN2
A3NANA$B1
B1ANANA$1
N1A$BANA2
N2ANA$BA3

BANANA$

$1BANANA1
A1$BANAN1
A2NA$BAN2
A3NANA$B1
B1ANANA$1
N1A$BANA2
N2ANA$BA3

# BWA-mem: Search for a pattern

We want to search for «ANA»

$\$_1$BANAN$A_1$
$A_1$\$BANA$N_1$
$A_2$NA\$BA$N_2$
$A_3$NANA\$$B_1$
$B_1$ANANA$\$_1$
$N_1$A\$BAN$A_2$
$N_2$ANA\$B$A_3$

$\$_1$BANAN$A_1$
$A_1$\$BANA$N_1$
$A_2$NA\$BA$N_2$
$A_3$NANA\$$B_1$
$B_1$ANANA$\$_1$
$N_1$A\$BAN$A_2$
$N_2$ANA\$B$A_3$

We have two occurences but we don't know where are the genome!

Suffix Array: each row corresponds to the path to the «$»

1 2 3 4 5 6 7
B A N A N A $

7 $\$_1$BANAN$A_1$
$A_1$\$BANA$N_1$
$A_2$NA\$BA$N_2$
$A_3$NANA\$$B_1$
$B_1$ANANA$\$_1$
$N_1$A\$BAN$A_2$
$N_2$ANA\$B$A_3$

1 2 3 4 5 6 7
B A N A N A $

7 $\$_1$BANAN$A_1$
6 $A_1$\$BANA$N_1$
$A_2$NA\$BA$N_2$
$A_3$NANA\$$B_1$
$B_1$ANANA$\$_1$
$N_1$A\$BAN$A_2$
$N_2$ANA\$B$A_3$

...

7 $\$_1$BANAN$A_1$
6 $A_1$\$BANA$N_1$
4 $A_2$NA\$BA$N_2$
2 $A_3$NANA\$$B_1$
1 $B_1$ANANA$\$_1$
5 $N_1$A\$BAN$A_2$
3 $N_2$ANA\$B$A_3$

ANA occurs in positions 4 and 2!

# Connect to server

1. Enter in the server:
   a. ssh lessons@157.27.80.26
   b. Password: lez2021
2. Enter in the created folder: cd HGE_2021/your_name

# Alignment with BWA command

- Align your reads to the reference genome (chr6 hg38):

```
/opt/bwa/bwa mem /home/lessons/HGE_2021/ref/chr6.hg38.fa
trimmed1.fastq.gz trimmed2.fastq.gz > sample.bwa.sam
```

- Turn your file sam into file bam:

```
samtools view -bT /home/lessons/HGE_2021/ref/chr6.hg38.fa -o sample.bwa.bam
sample.bwa.sam
```

# Alignment with BWA command

- Sort your file:

  samtools sort sample.bwa.bam -o sample.sorted.bwa.bam

- Create index for your bam file:

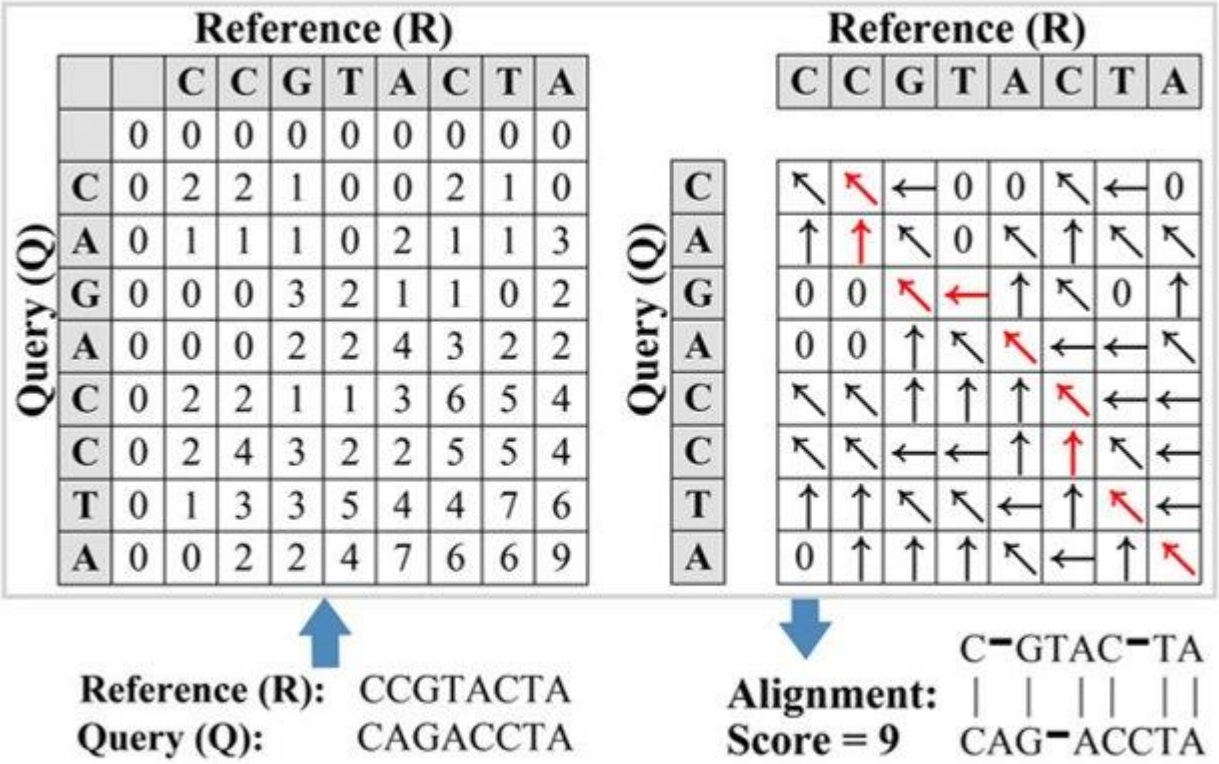
  samtools index sample.sorted.bwa.bam

# Isaac4

**Isaac4** is a sequence alignment and variant detector tool developed by Illumina based on Smith-Waterman algorithm. Isaac employs a large amount of memory to produce ultrafast alignments and is reportedly 45 times faster than the BWA pipeline. **It is used especially for DNA sequencing data: targeting sequencing, WES, WGS. It breaks reads up to 10,000bp. Isaac soft-clip unmapped bases and allows to soft-clip overlapping reads using the option --clip-overlapping 1.**

## Smith-Waterman algorithm

The Smith-Waterman algorithm compare the reference to the query using local alignment.




match=+2
mismatch=-1
gap=1

# Isaac4: construct matrix

We search for the pattern TCC in our reference genome sequence AATCC

Example:
match= +2
mismatch= -1
gap= -1

| | | A | A | T | C | C |
|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | | | | | |
| C | 0 | | | | | |
| C | 0 | | | | | |

1. In the first step, the first row and column are set to 0

| | | A | A | T | C | C |
|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | | | | | |
| C | 0 | | | | | |
| C | 0 | | | | | |

2. We want to fill the first cell. To do that we have to check all the possibilities from the 3 cells before.

| | | A |
|---|---|---|
| | 0 | 0 |
| T | 0 | |

Gap: -1

Mismatch: -1

Gap: -1

So the max is 1, so the value of the cell is 1.

# Isaac4: construct matrix

We search for the pattern TCC in our reference genome sequence AATCC

Example:
match= +2
mismatch= -1
gap= -1

| | | A | A | T | C | C |
|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | 1 | | | | |
| C | 0 | | | | | |
| C | 0 | | | | | |

1. In the first step, the first row and column are set to 0

| | | A | A |
|---|---|---|---|
| | 0 | 0 | 0 |
| T | 0 | -1 | |

Gap: 0 + 1 = -1

Mismatch: 0-1 = -1

Gap: -1-1 = -2

So the max is 2, so the value of the cell is 2.

| | | A | A | T | C | C |
|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | -1 | -1 | 2 | 1 | 0 |
| C | 0 | -1 | -2 | 1 | 4 | 3 |
| C | 0 | -1 | -2 | 0 | 5 | 4 |

# Isaac4: reconstruct the path

We search for the pattern TCC in our reference genome sequence AATCC

| | | A | A | T | C | C |
|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | -1 | -1 | 2 | 1 | 0 |
| C | 0 | -1 | -2 | 1 | 4 | 3 |
| C | 0 | -1 | -2 | 0 | 5 | 8 |

Reconstruct starting from the highest value, back up to the start

The local alignment will be:

AATCC

- - TCC

# Alignment with Isaac4 command

- Create a folder: mkdir Fastq
- Copy fastq files into the folder:

```
cp R1.fastq.gz Fastq/lane1_read1.fastq.gz
cp R2.fastq.gz Fastq/lane1_read2.fastq.gz
```

- Align your reads to the reference genome (chr6 hg38):

```
/opt/Isaac4/bin/isaac-align -r /home/lessons/HGE_2021/ref/chr6.hg38.fa -b $(pwd) --base-calls-format fastq -t Temp -o Aligned --default-adapters Standard -m30 --keep-duplicates 0 -j 5 --clip-overlapping 1
```

# Alignment command

- Open the **BWA** file:

  samtools view sample.sorted.bwa.bam| less –S

- Close the visualization: q

# Alignment output – BAM file

Header

Body

```
@HD     VN:1.3  SO:coordinate
@SQ     SN:chr2 LN:243199373
@PG     ID:bwa  PN:bwa  VN:0.7.15-r1140 CL:bwa mem chr2.fasta read1.trimmed.fastq.gz read2.trimmed.fastq.gz
NB500897:75:H5NWWBGXY:3:13608:21224:10540       2147    chr2    12323980        0       57H38M21H       =       69870261        57546346        GGCTGAGGTGGGAG
NB500897:78:H5T5FBGXY:4:11401:24478:5392        2131    chr2    25275067        0       108H33M9H       =       69650885        44375787        AAAAATAAAAATAA
NB500897:75:H5NWWBGXY:3:12601:23290:7800        2115    chr2    33141345        0       94H56M  =       68269866        35128522        GGGGGGGGGGGGGGGGGGGGGG
NB500897:78:H5T5FBGXY:3:12609:16449:18291       2115    chr2    33141438        0       120H30M =       68547068        35405631        GGGGGGGGGGGGGGGGGGGGGG
NB500897:75:H5NWWBGXY:1:13104:6732:16100        2115    chr2    33141478        0       120H30M =       68547068        35405591        GGGGGGGGGGGGGGGGGGGGGG
NB500897:78:H5T5FBGXY:4:12411:14105:19392       2147    chr2    33141574        0       100H50M =       69871302        36729810        GGGGGGGGGGGGGGGGGGGGGG
NB500897:75:H5NWWBGXY:2:23311:23906:6768        2115    chr2    33141581        0       87H43M  =       68269811        35128231        GGGGGGGGGGGGGGGGGGGGGG
NB500897:75:H5NWWBGXY:1:12106:7875:15761        2115    chr2    33141594        0       120H30M =       68547068        35405475        GGGGGGGGGGGGGGGGGGGGGG
NB500897:78:H5T5FBGXY:3:22601:12050:10673       161     chr2    50626928        0       2S64M   =       69890571        19263716        AAAAAAAAAAAAAAAAAAAAAA
NB500897:75:H5NWWBGXY:4:12607:26302:14475       81      chr2    52194430        0       78M     =       69688660        17494154        ATGTTGGCGAGGCTGGTCTCCA
NB500897:78:H5T5FBGXY:4:23607:18581:7865        163     chr2    58355595        0       26M     =       58355665        151     TAGCTGGGATTACAGGTGTGTGCCAC
NB500897:78:H5T5FBGXY:4:23607:18581:7865        83      chr2    58355665        0       4S81M13S        =       58355595        -151    CTCACCATGTTGCCCAGGCTGG
NB500897:75:H5NWWBGXY:3:13608:21224:10540       2179    chr2    65437823        0       63M53H  =       69870261        4432439 ACTATGCTGACCAGGTTGGTTTCAAATTCC
NB500897:75:H5NWWBGXY:2:11103:13114:1410        99      chr2    67999995        60      66M     =       68000128        259     CTCACAATAAATTTATTTTTTCAAAAGCAG
NB500897:75:H5NWWBGXY:2:11103:13114:1410        147     chr2    68000128        60      126M    =       67999995        -259    CATCTAGATAGCTATCTTTCCAGACTTTTC
NB500897:78:H5T5FBGXY:1:21201:19536:13168       99      chr2    68000446        60      150M    =       68000452        156     CCTCAGAGTATTAAAGACCACATAGTATAT
NB500897:78:H5T5FBGXY:4:23607:24808:1972        99      chr2    68000446        60      150M    =       68000452        156     CCTCAGAGTATTAAAGACCACATAGTATAT
NB500897:78:H5T5FBGXY:1:21201:19536:13168       147     chr2    68000452        60      150M    =       68000446        -156    AGTATTAAAGACCACATAGTATATATTTTC
NB500897:78:H5T5FBGXY:4:23607:24808:1972        147     chr2    68000452        60      150M    =       68000446        -156    AGTATTAAAGACCACATAGTATATATTTTC
NB500897:75:H5NWWBGXY:4:12408:9893:6566 163     chr2    68001096        60      150M    =       68001096        150     AGATTCCCTGGCTAAATTCCACCATTGAAAGAAATTGA
NB500897:75:H5NWWBGXY:4:12408:9893:6566 83      chr2    68001096        60      150M    =       68001096        -150    AGATTCCCTGGCTAAATTCCACCATTGAAAGAAATTGA
NB500897:78:H5T5FBGXY:2:12107:7865:18634        99      chr2    68001412        60      150M    =       68001549        287     TTCCTAACTAAATACTGACTAGAACAGTGA
NB500897:78:H5T5FBGXY:2:12107:7865:18634        147     chr2    68001549        60      150M    =       68001412        -287    CCCAACTCCTGCCACTCTAGCCACATCAAG
NB500897:78:H5T5FBGXY:1:12109:14488:19284       99      chr2    68001927        60      150M    =       68002195        418     ACGTAGTAGAAATTCACAGAATACTTGGTA
NB500897:78:H5T5FBGXY:1:12305:16077:11340       163     chr2    68001976        60      150M    =       68002289        462     AATTCCTCACCAGCTTCAGCAGCTTAAGGA
NB500897:75:H5NWWBGXY:1:22202:17880:17856       99      chr2    68001995        60      150M    =       68002066        221     CAGCTTAAGGATAAAGAATCTTGCATCTAA
```

Read ID

Mapping Tag

Mapping position

Mapping quality

CIGAR string

Pair information

https://samtools.github.io/hts-specs/SAMv1.pdf

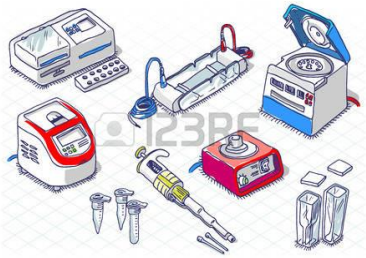
# Cigar

| Op | Description |
|---|---|
| M | alignment match (can be a sequence match or mismatch) |
| I | insertion to the reference |
| D | deletion from the reference |
| N | skipped region from the reference |
| S | soft clipping (clipped sequences present in SEQ) |
| H | hard clipping (clipped sequences NOT present in SEQ) |
| P | padding (silent deletion from padded reference) |
| = | sequence match |
| X | sequence mismatch |

# DUPLICATES REMOVAL

Library preparation

Bioinformatic analysis



Sequencing

Data QC → Alignment → Variant calling → Variant annotation → Variant prioritization

# Picard Mark Duplicates Algorithm

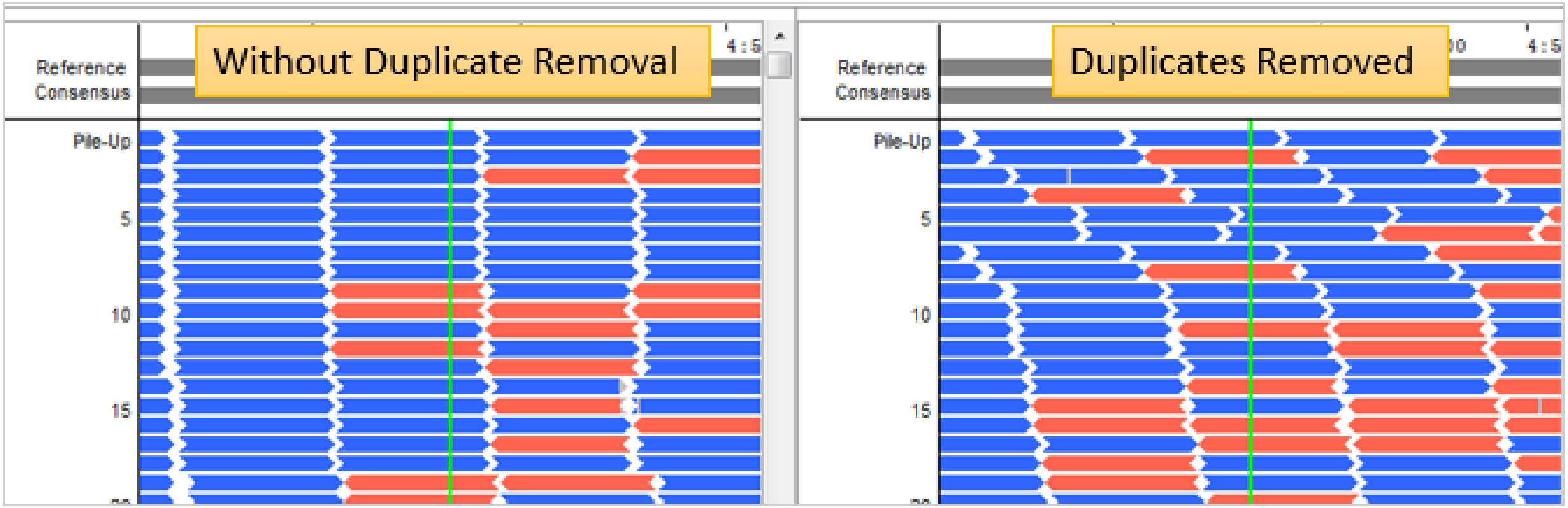
**Picard by GATK:**

The MarkDuplicates tool works by comparing sequences in the 5 prime positions of both reads and read-pairs in a SAM/BAM file. After duplicate reads are collected, the tool differentiates the primary and duplicate reads using an algorithm that ranks reads by the sums of their base-quality scores (default method).

gatk

BROAD INSTITUTE

# Picard Mark Duplicates



# Mark Duplicates

1. Mark Duplicates with picard:

```
java -jar /opt/picard.jar MarkDuplicates
INPUT=sample.sorted.bwa.bam
OUTPUT=sample.sorted.dedup.bwa.bam
REMOVE_DUPLICATES=false METRICS_FILE=duplicates.txt
```

2. Open the output file: less –S duplicates.txt

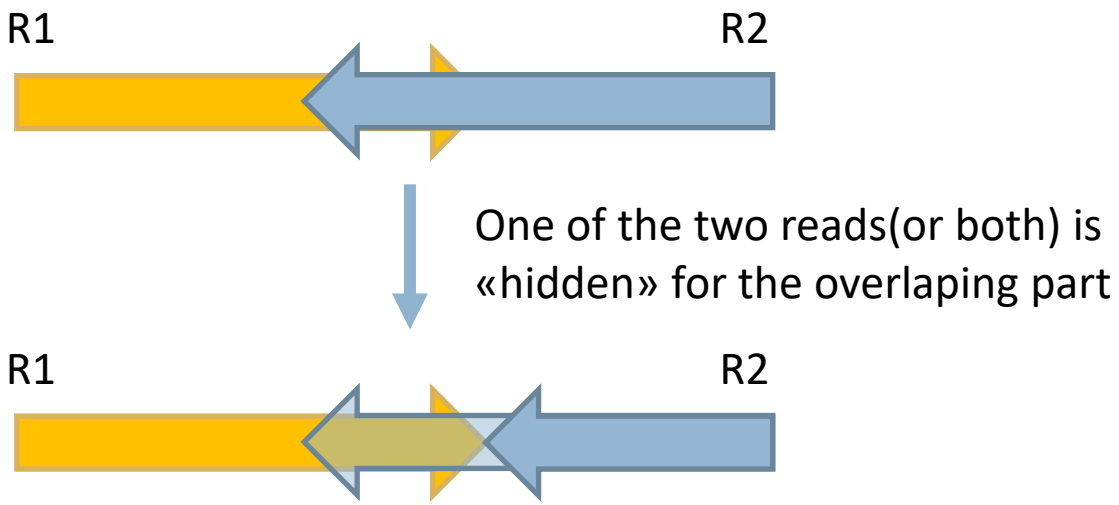
```
## htsjdk.samtools.metrics.StringHeader
# MarkDuplicates INPUT=[sample.sorted.bwa.bam] OUTPUT=sample.sorted.dedup.bwa.bam METRICS_FILE=duplicates.txt REMOVE_DUPLICATES=false
## htsjdk.samtools.metrics.StringHeader
# Started on: Wed Jan 13 14:22:59 CET 2021

## METRICS CLASS        picard.sam.DuplicationMetrics
LIBRARY UNPAIRED_READS_EXAMINED READ_PAIRS_EXAMINED     SECONDARY_OR_S[illegible]_RDS  UNMAPPED_READS  UNPAIRED_READ_DUPLICATES
Unknown Library 1305    2220416 32743   1355    553     211540  16339   0,095367        11697388
```

Percentage of duplicates

# READ CLIPPING

# BamUtils clipping

**Bamutil**: The clipOverlap option of bamUtil **only soft-clips** overlapping read pairs. When two mates overlap, **this tool will clip the record's whose clipped region would have the lowest average quality**.



# BamUtils clipping command

1. Soft-clip overlapping reads with BamUtils:

/opt/bamUtil/bin/bam clipOverlap --in sample.sorted.dedup.bwa.bam --out sample.sorted.dedup.clipped.bwa.bamUtils.bam

2. Create index:

samtools index sample.sorted.dedup.clipped.bwa.bamUtils.bam

View bam **before** soft-clipping:

samtools view sample.sorted.dedup.bwa.bam | less -S

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MG01HX01:853:HWY5YCCXY:5:2120:11627:31336 | 99 | chr6 | 60065 | 60 | 151M | = | 60223 | 309 |
| MG01HX01:853:HWY5YCCXY:5:2120:11627:31336 | 147 | chr6 | 60223 | 60 | 151M | = | 60065 | -309 |
| MG01HX01:853:HWY5YCCXY:3:2215:26839:21649 | 99 | chr6 | 61798 | 60 | 151M | = | 61908 | 261 |
| MG01HX01:853:HWY5YCCXY:3:2215:26839:21649 | 147 | chr6 | 61908 | 60 | 151M | = | 61798 | -261 |
| MG01HX01:853:HWY5YCCXY:6:1104:18213:52731 | 163 | chr6 | 62841 | 60 | 151 | = | 63114 | 424 |

CIGAR string has been changed, 41 bases were soft-clipped

View bam **after** soft-clipping:

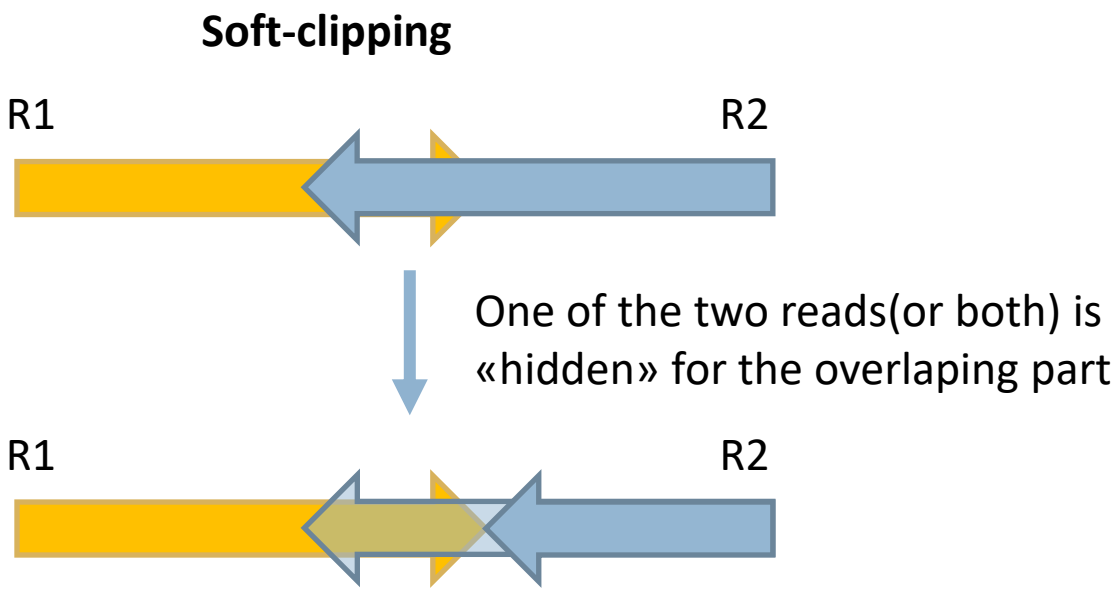
samtools view sample.sorted.dedup.clipped.bwa.bamUtils.bam | less -S

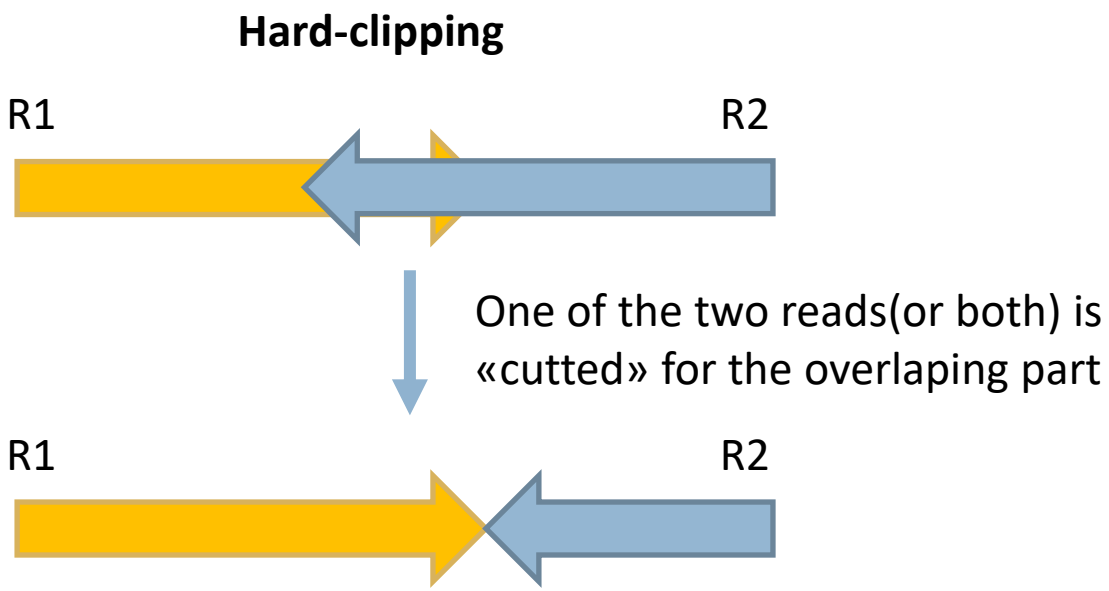
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MG01HX01:853:HWY5YCCXY:5:2120:11627:31336 | 99 | chr6 | 60065 | 60 | 151M | = | 60223 | 309 |
| MG01HX01:853:HWY5YCCXY:5:2120:11627:31336 | 147 | chr6 | 60223 | 60 | 151M | = | 60065 | -309 |
| MG01HX01:853:HWY5YCCXY:3:2215:26839:21649 | 99 | chr6 | 61798 | 60 | 110M41S | = | 61908 | 261 |
| MG01HX01:853:HWY5YCCXY:3:2215:26839:21649 | 147 | chr6 | 61908 | 60 | 151M | = | 61798 | -261 |
| MG01HX01:853:HWY5YCCXY:6:1104:18213:52731 | 163 | chr6 | 62841 | 60 | 151M | = | 63114 | 424 |

# fgBio clipping

Fgbio ClipBam: Clips overlapping read pairs from the same fragment. **Three clipping modes** are supported:

- **Soft:** soft-clip the bases and qualities.
- **SoftWithMask:** soft-clip and mask the bases and qualities (make bases Ns and qualities the minimum).
- **Hard (default):** hard-clip the bases and qualities.



# fgBio clipping command

1. Hard-clip overlapping reads with BamUtils:

```
java -jar /opt/fgbio-1.1.0.jar  ClipBam -i sample.sorted.dedup.bwa.bam -o sample.sorted.dedup.bwa.fgbio.bam -r
../ref/chr6.hg38.fa -c Hard --clip-overlapping-reads true
```

View bam **before** hard-clipping:

```
samtools view sample.sorted.dedup.bwa.bam | less -S
```

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MG01HX01:853:HWY5YCCXY:5:2120:11627:31336 | 99 | chr6 | 60065 | 60 | 151M | = | 60223 | 309 |
| MG01HX01:853:HWY5YCCXY:5:2120:11627:31336 | 147 | chr6 | 60223 | 60 | 151M | = | 60065 | -309 |
| MG01HX01:853:HWY5YCCXY:3:2215:26839:21649 | 99 | chr6 | 61798 | 60 | 151M | = | 61908 | 261 |
| MG01HX01:853:HWY5YCCXY:3:2215:26839:21649 | 147 | chr6 | 61908 | 60 | 151M | = | 61798 | -261 |
| MG01HX01:853:HWY5YCCXY:6:1104:18213:52731 | 163 | chr6 | 62841 | 60 | 151 | = | 63114 | 424 |

CIGAR string has been changed, 41 bases were soft-clipped from the two paires

View bam **after** hard-clipping:

```
samtools view sample.sorted.dedup.clipped.bwa.fgbio.bam | less -S
```

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MG01HX01:853:HWY5YCCXY:5:2120:11627:31336 | 99 | chr6 | 60065 | 60 | 151M | = | 60223 | 309 |
| MG01HX01:853:HWY5YCCXY:5:2120:11627:31336 | 147 | chr6 | 60223 | 60 | 151M | = | 60065 | -309 |
| MG01HX01:853:HWY5YCCXY:3:2215:26839:21649 | 99 | chr6 | 61798 | 60 | 131M20H | = | 61929 | 261 |
| MG01HX01:853:HWY5YCCXY:3:2215:26839:21649 | 147 | chr6 | 61929 | 60 | 21H130M | = | 61798 | -261 |
| MG01HX01:853:HWY5YCCXY:6:1104:18213:52731 | 163 | chr6 | 62841 | 60 | 151M | = | 63114 | 424 |

# Insert size

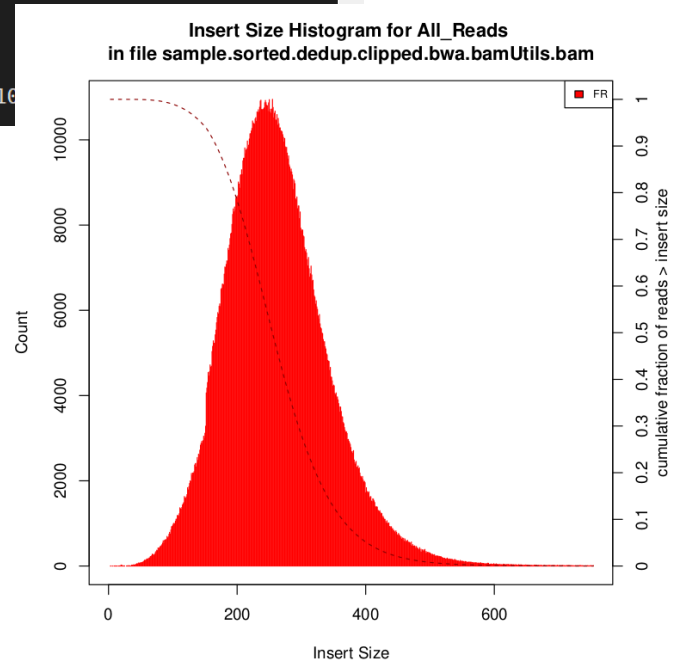
1. Calculate the insert size using picard:

```
java -jar /opt/picard.jar CollectInsertSizeMetrics I=sample.sorted.dedup.clipped.bwa.bamUtils.bam
H=sample.sorted.dedup.clipped.bwa.bamUtils.hist.pdf O=sample.sorted.dedup.clipped.bwa.bamUtils.output AS=true
VALIDATION_STRINGENCY=SILENT
```

2. Check the results:

```
less -S sample.sorted.dedup.clipped.bwa.bamUtils.output
```

```
## htsjdk.samtools.metrics.StringHeader
# CollectInsertSizeMetrics HISTOGRAM_FILE=sample.sorted.dedup.clipped.bwa.bamUtils.hist.pdf INPUT=sample.sorted.dedup.clipped.bwa.bamUtils.bam OUTPUT=sampl
## htsjdk.samtools.metrics.StringHeader
# Started on: Wed Jan 13 15:24:07 CET 2021

## METRICS CLASS        picard.analysis.InsertSizeMetrics
MEDIAN_INSERT_SIZE      MODE_INSERT_SIZE        MEDIAN_ABSOLUTE_DEVIATION       MIN_INSERT_SIZE MAX_INSERT_SIZE MEAN_INSERT_SIZE
254     255     50      3       170533062       261,069854      79,617853       1977539 FR      19      39      57      79      16
```



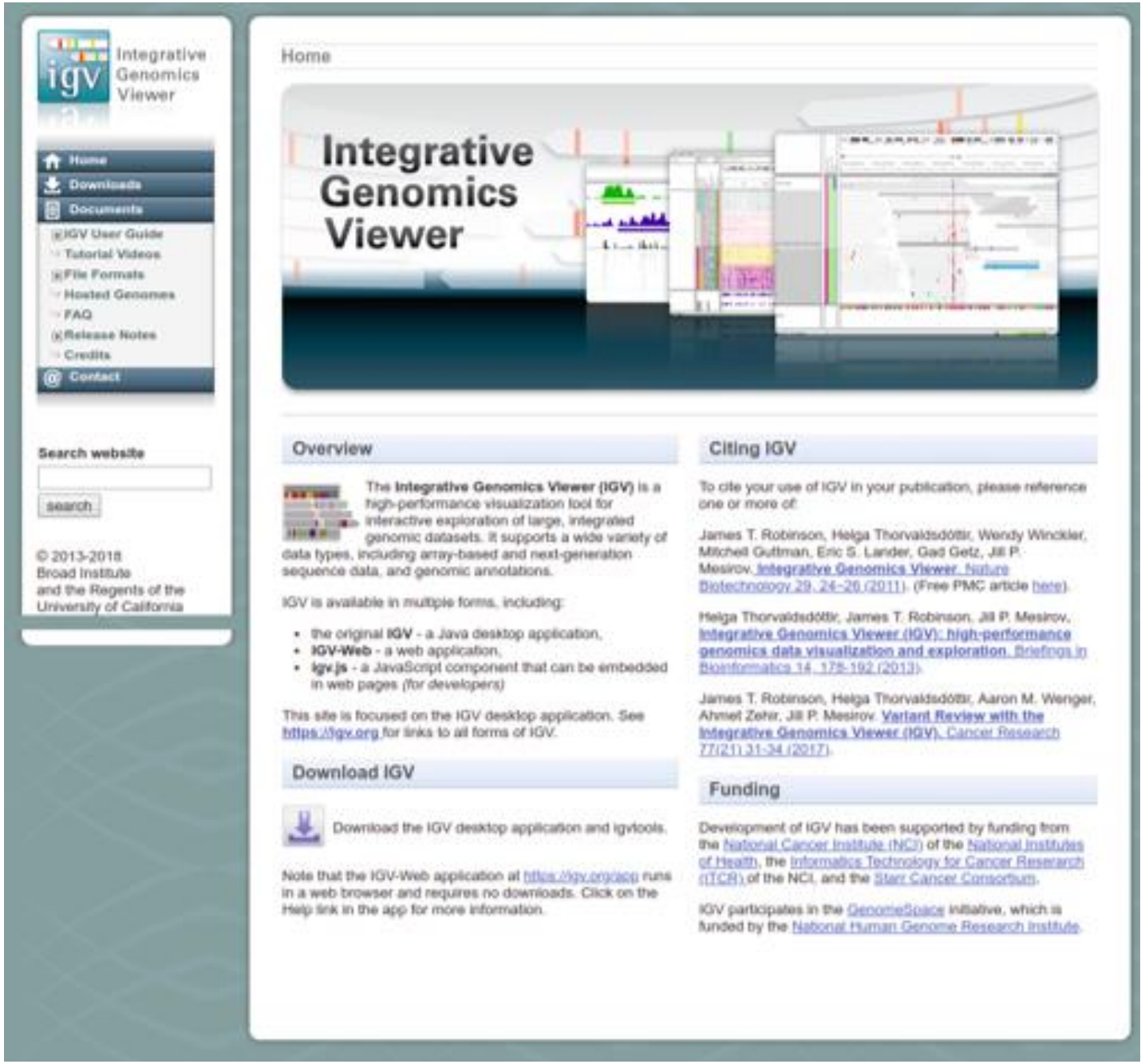

3. Check the on histogram:

```
evince sample.sorted.dedup.clipped.bwa.bamUtils.hist.pdf
```

# VISUALIZATION OF ALIGNED READS ON IGV

# IGV

https://software.broadinstitute.org/software/igv/



# Download IGV

## Install IGV 2.8.x

See the Release Notes for what's new in each release.

### IGV Mac App

Download and unzip the Mac App Archive, then double-click the IGV application to run it.
You can move the app to the *Applications* folder, or anywhere else.

**MacOS Catalina users:** We sign our Mac App as a trusted Apple developer, but it is not yet notarized by Apple (a new requirement in Catalina). To run it, right-click on the downloaded IGV app; select "Open" from the menu; and click the "Open" button in the window that pops up. After that, double-clicking on the app will also work.

### IGV for Windows

Download and run the installer.
An IGV shortcut will be created on the Desktop; double-click it to run the application.

### IGV for Linux

Download and unzip the Archive.
See the downloaded *readme.txt* for further instructions.

### IGV and igvtools to run on the command line (all platforms)

Download and unzip the Archive. ***Requires Java 11.***
See the downloaded *readme.txt* and *igvtools_readme.txt* for further instructions.

# Download the bam and the bai

- Download the bam file and the index file on your pc:
- Open new terminal:

cd Desktop/HGE_2021

rsync -auv lessons@157.27.80.26:/home/lessons/HGE_2021/your_name/sample.sorted.dedup.clipped.bwa.bamUtils.ba* .

rsync -auv lessons@157.27.80.26:/home/lessons/HGE_2021/denise/sample.sorted.dedup.clipped.bwa.fgbio.ba* .

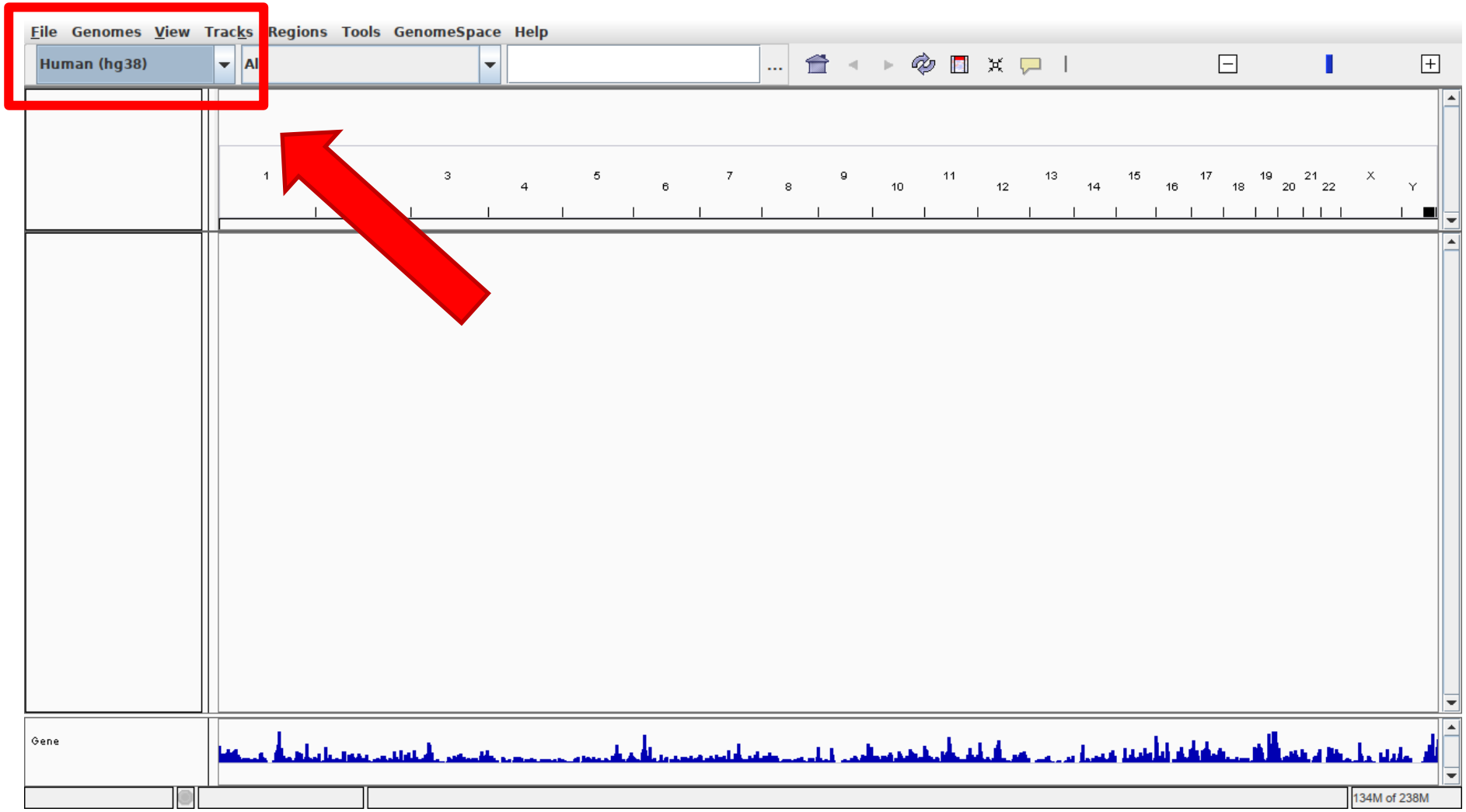
Password: lez2021

- Check if you have downloaded: ls

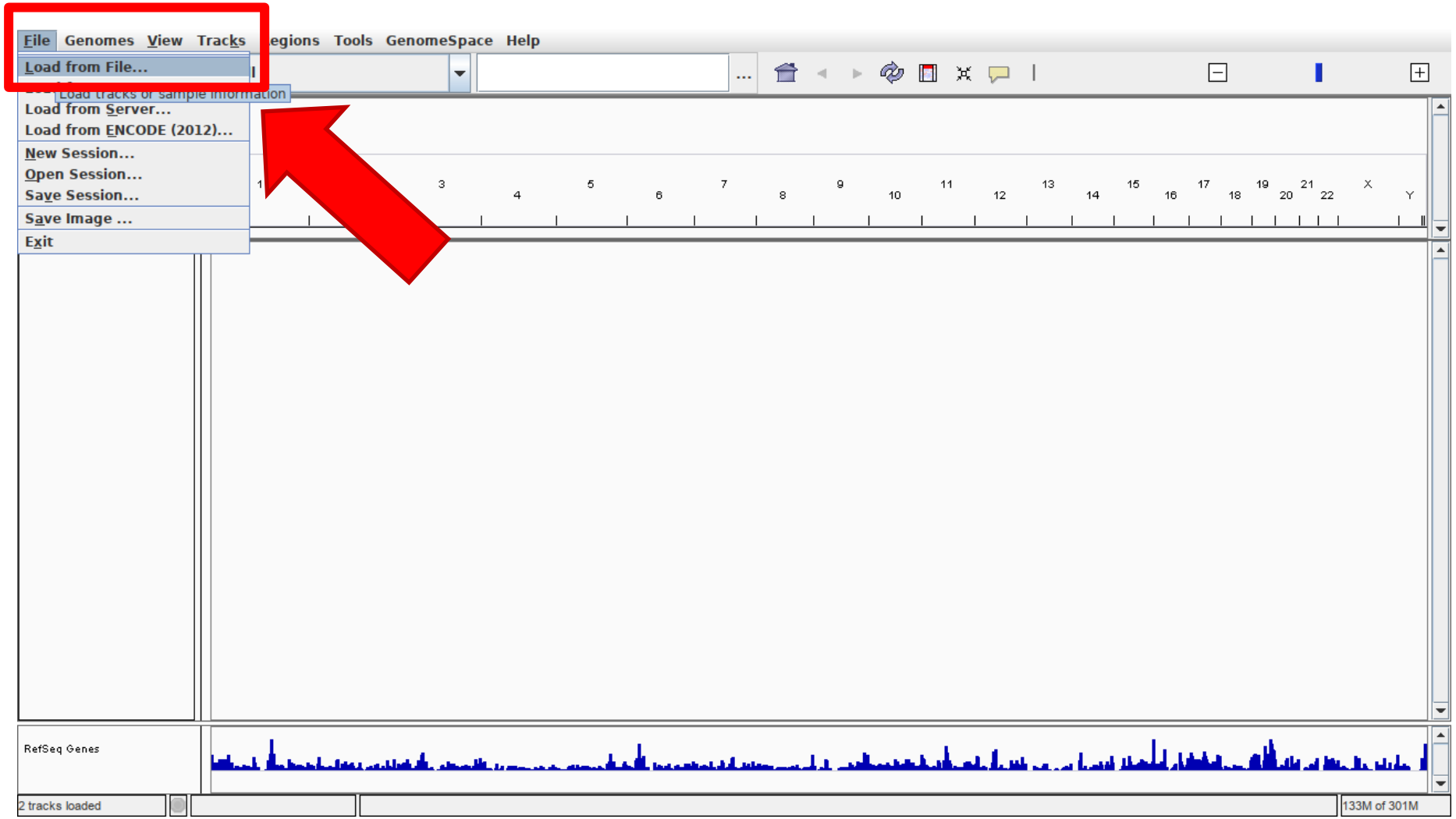
- Open IGV

  ./igv.sh for Ubuntu

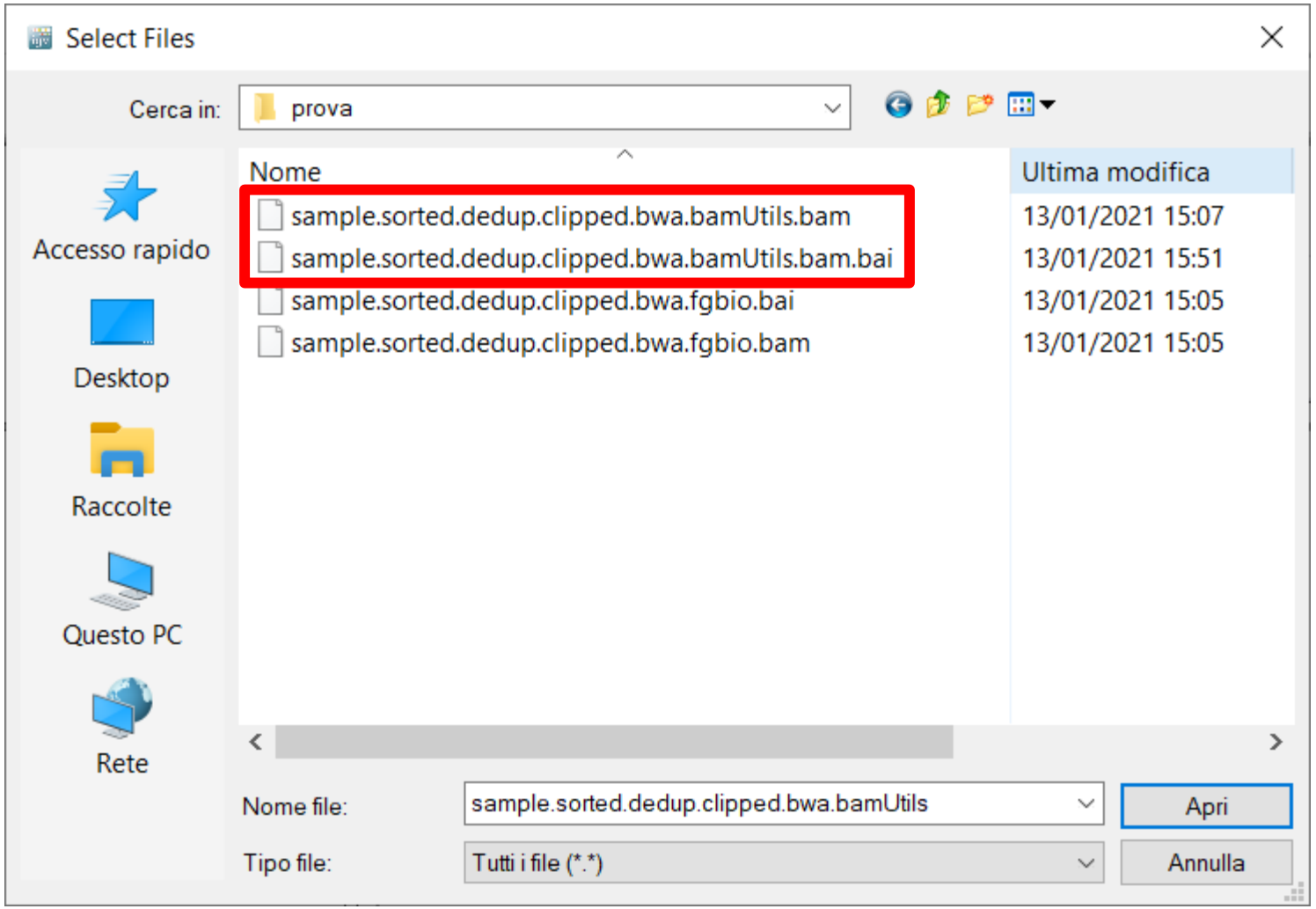
# Choose the right genome



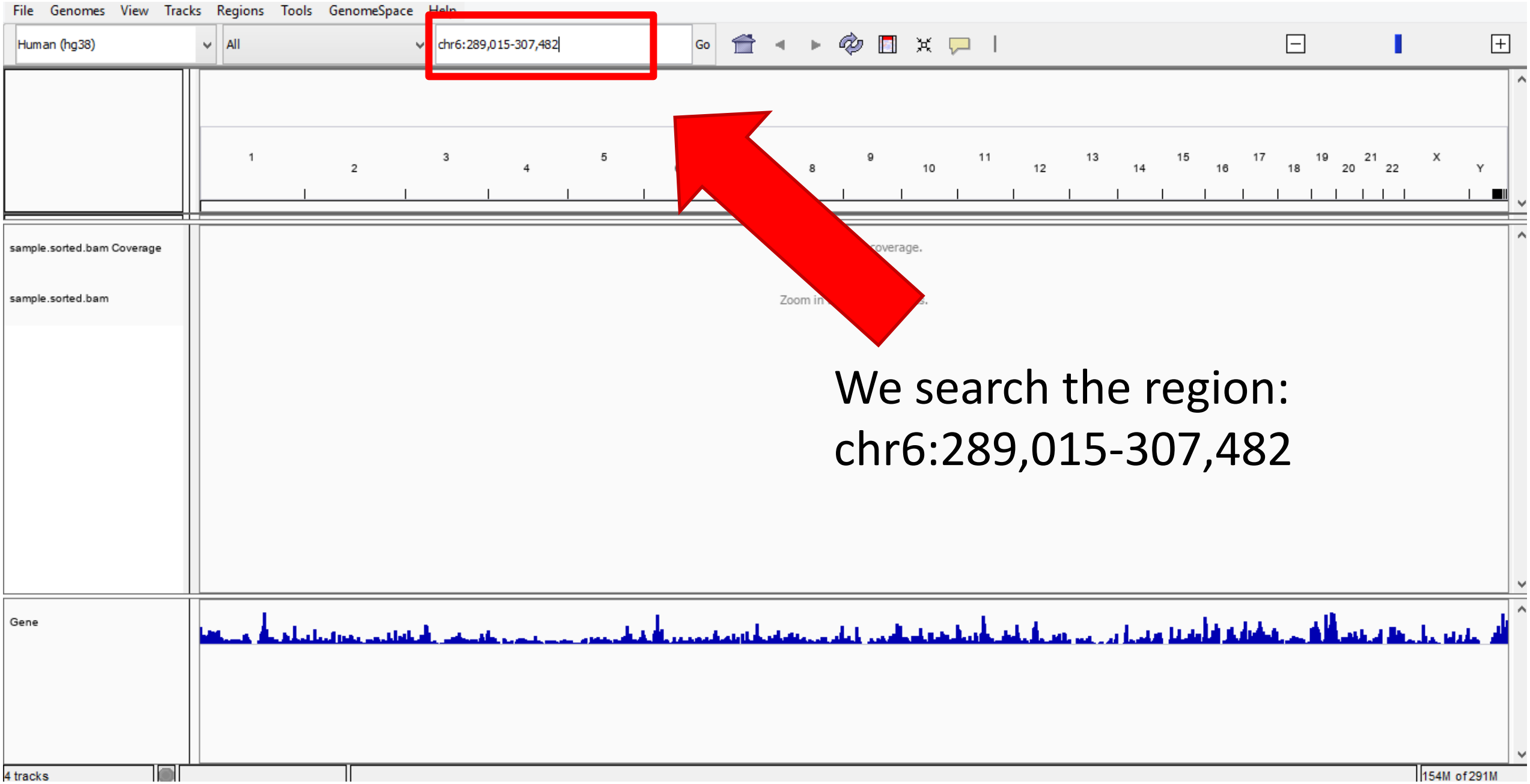
# Upload the bam



# Go into the folder «HGE_2021», choose the file bam and open it



# Search a specific region



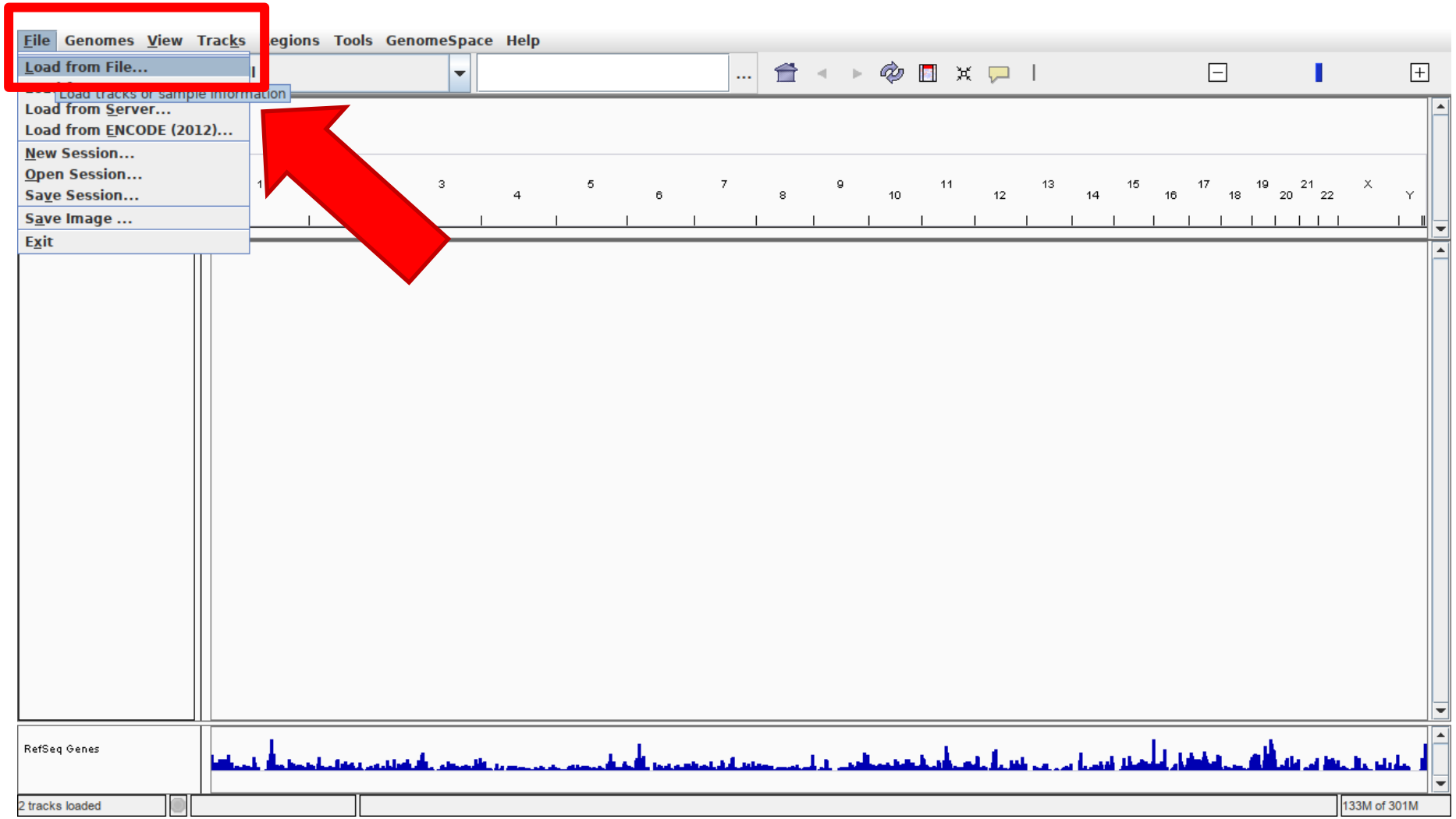

We search the region:
chr6:289,015-307,482

# Results

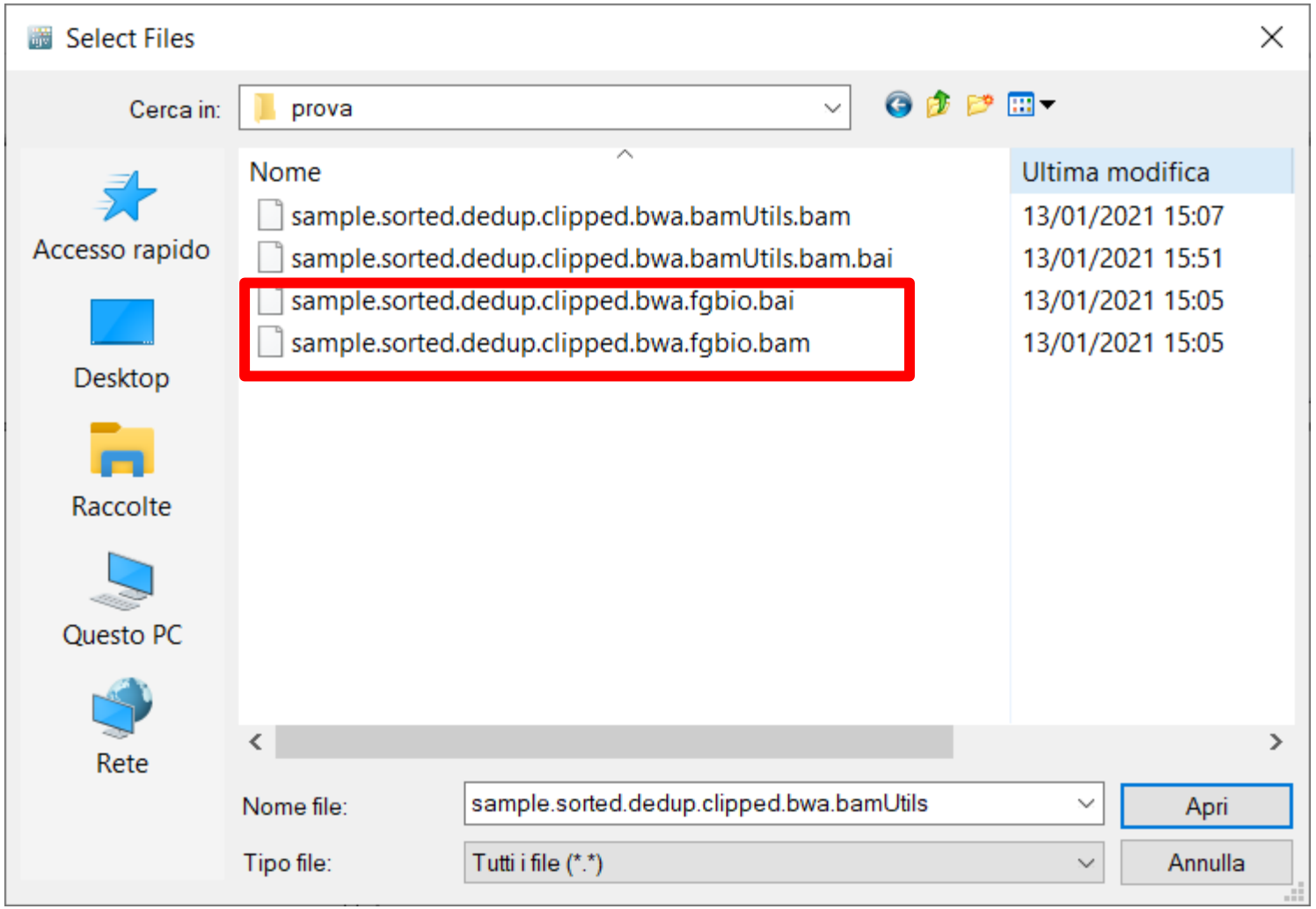

File Genomes View Tracks Regions Tools GenomeSpace Help
Human (hg38)
chr6
chr6:289,015-307,482
Go
p25.1 p24.1 p22.3 p22.2 p21.32 p21.1 p12.3 p12.1 q11.1 q13 q14.1 q14.3 q16.1 q16.3 q21 q22.1 q22.31 q22.33 q23.3 q24.2 q25.1 q25.3 q27
18 kb
290 kb 292 kb 294 kb 296 kb 298 kb 300 kb 302 kb 304 kb 306 kb
sample.sorted.bam Coverage
[0 - 146]
sample.sorted.bam
Gene
DUSP22

# Difference between genome and exome sequencing

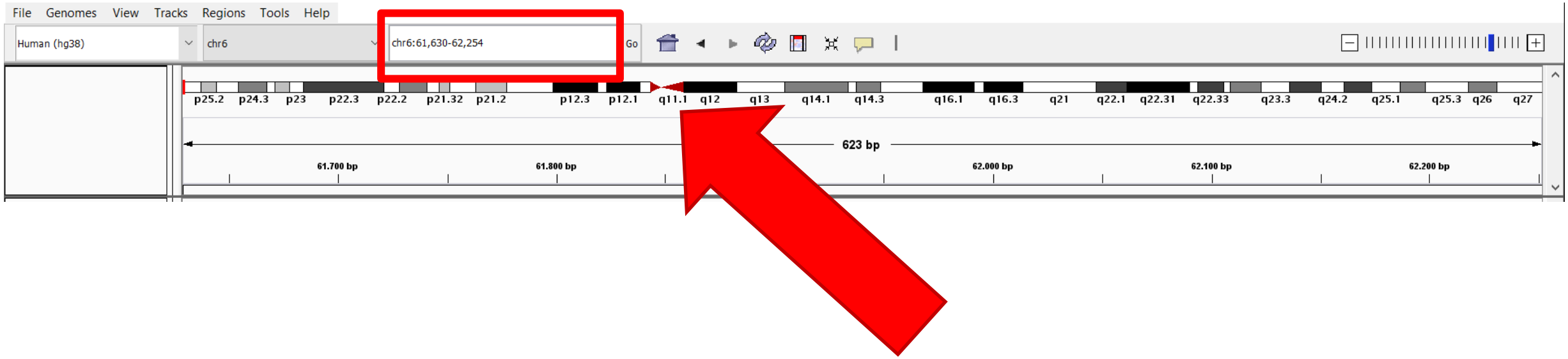
# Upload the second bam



# Go into the folder "HGSI2020", choose the file bam and open it

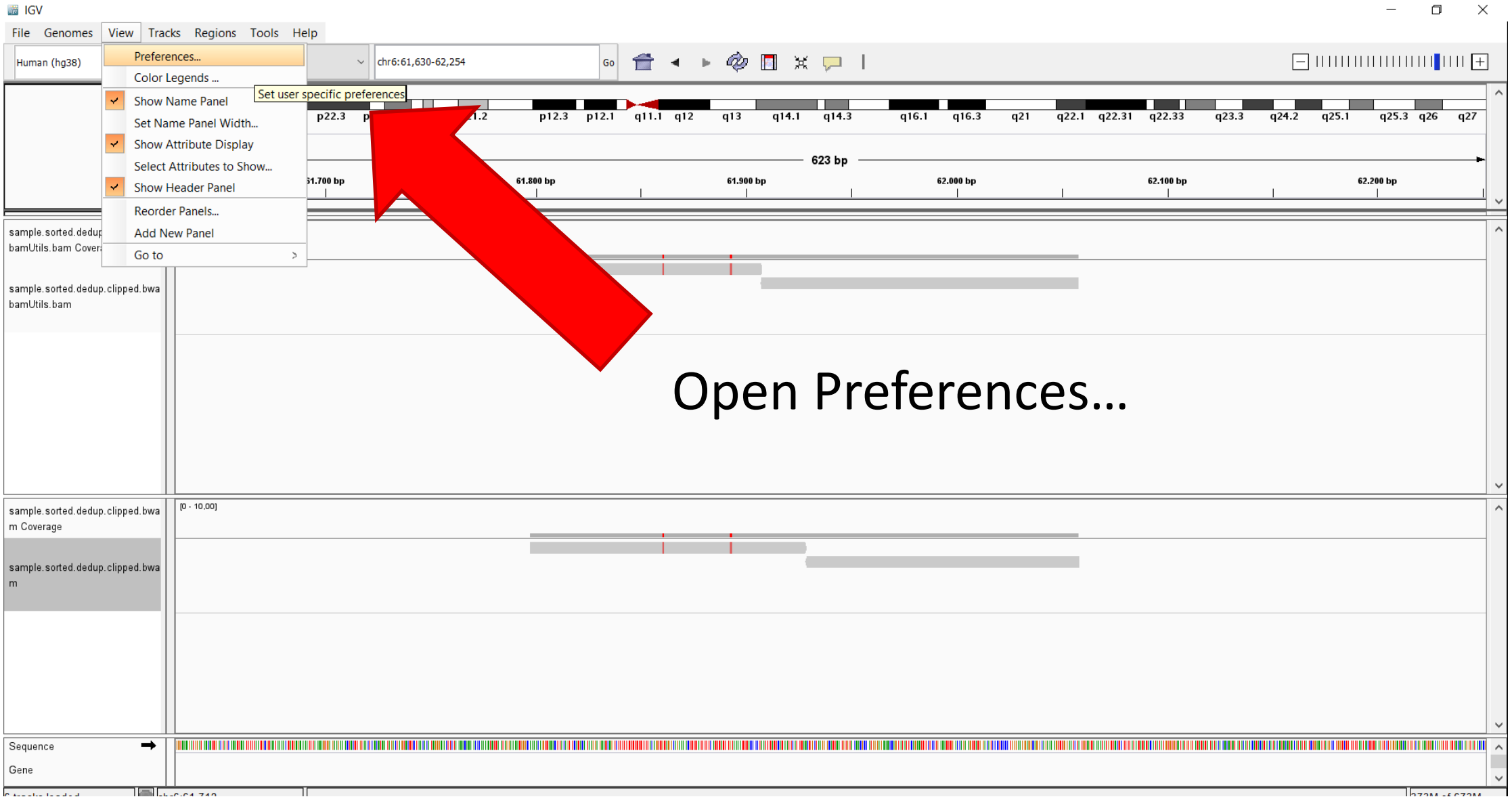


# Search a specific region



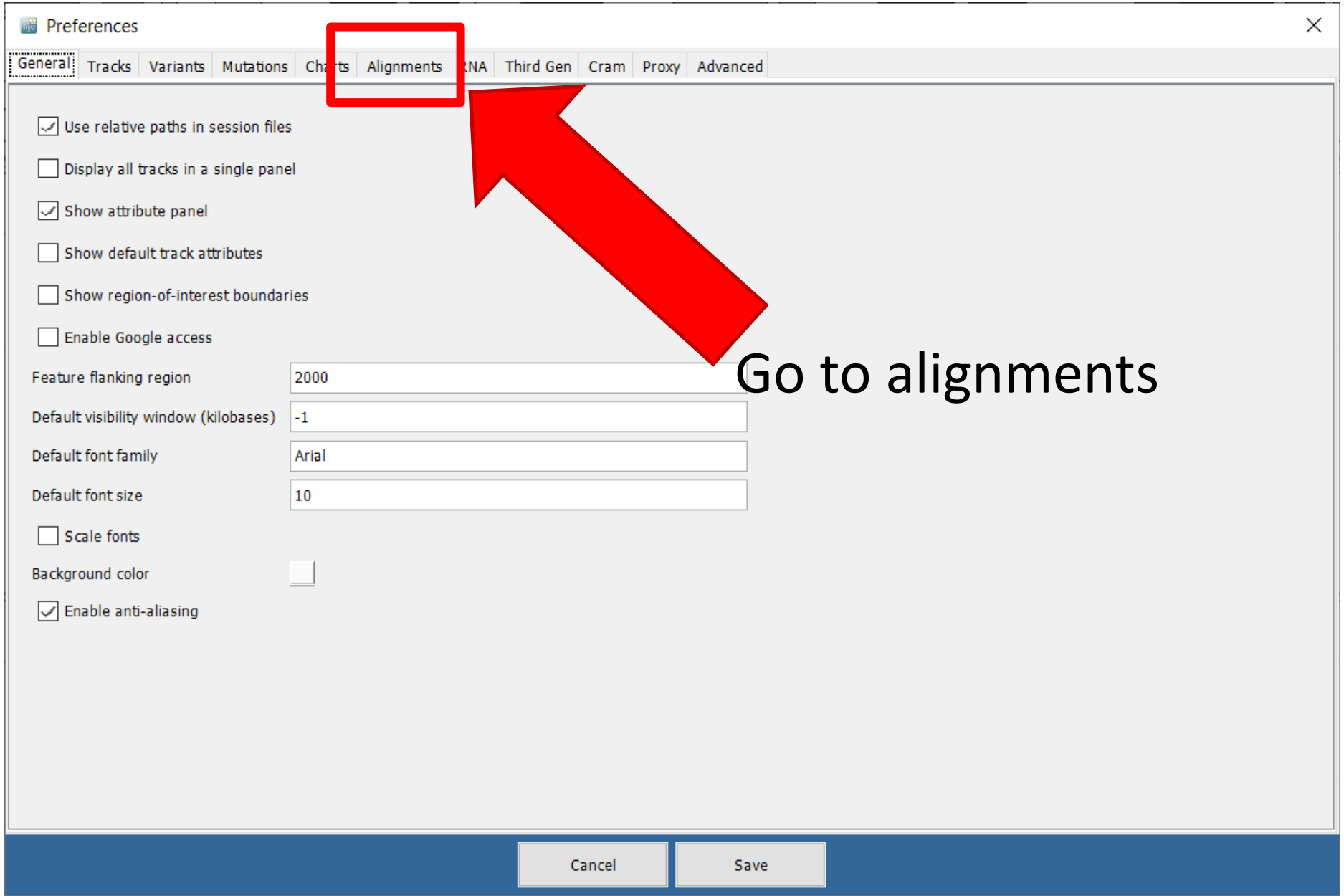

We search the region:
chr6:61,630-62,254

# Check differences between soft and hard clipping

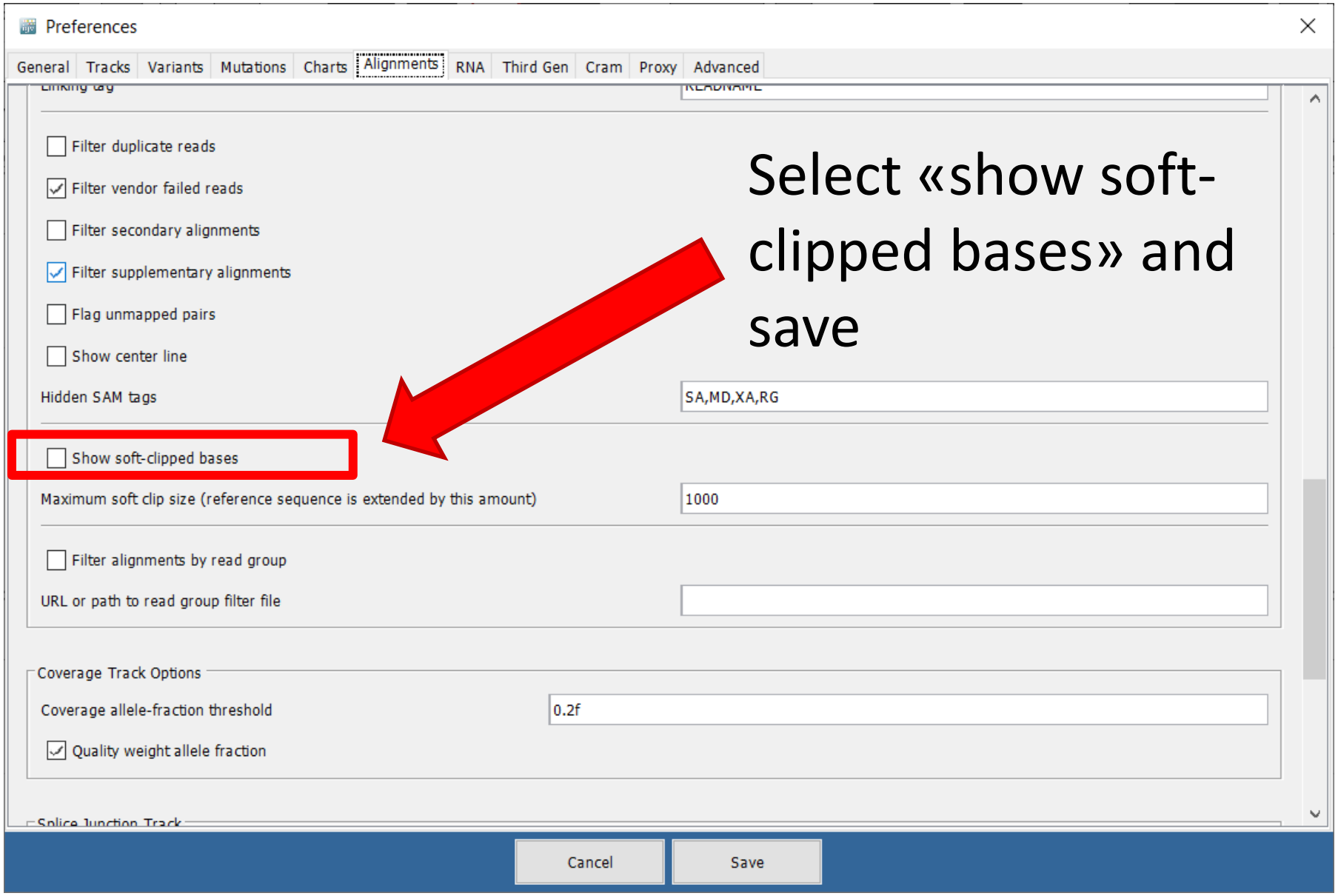



Open Preferences…

# Check differences between soft and hard clipping

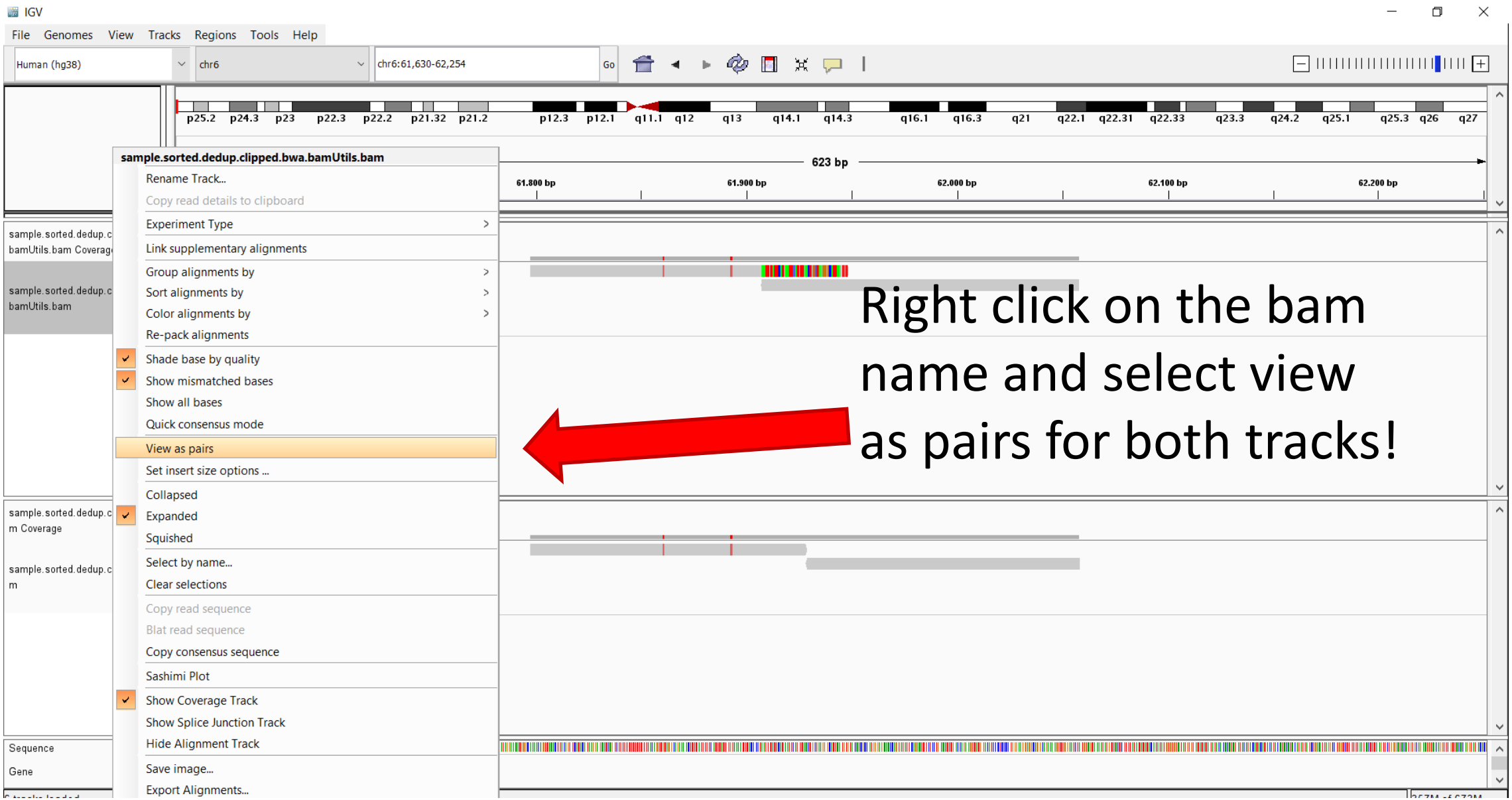


# Check differences between soft and hard clipping

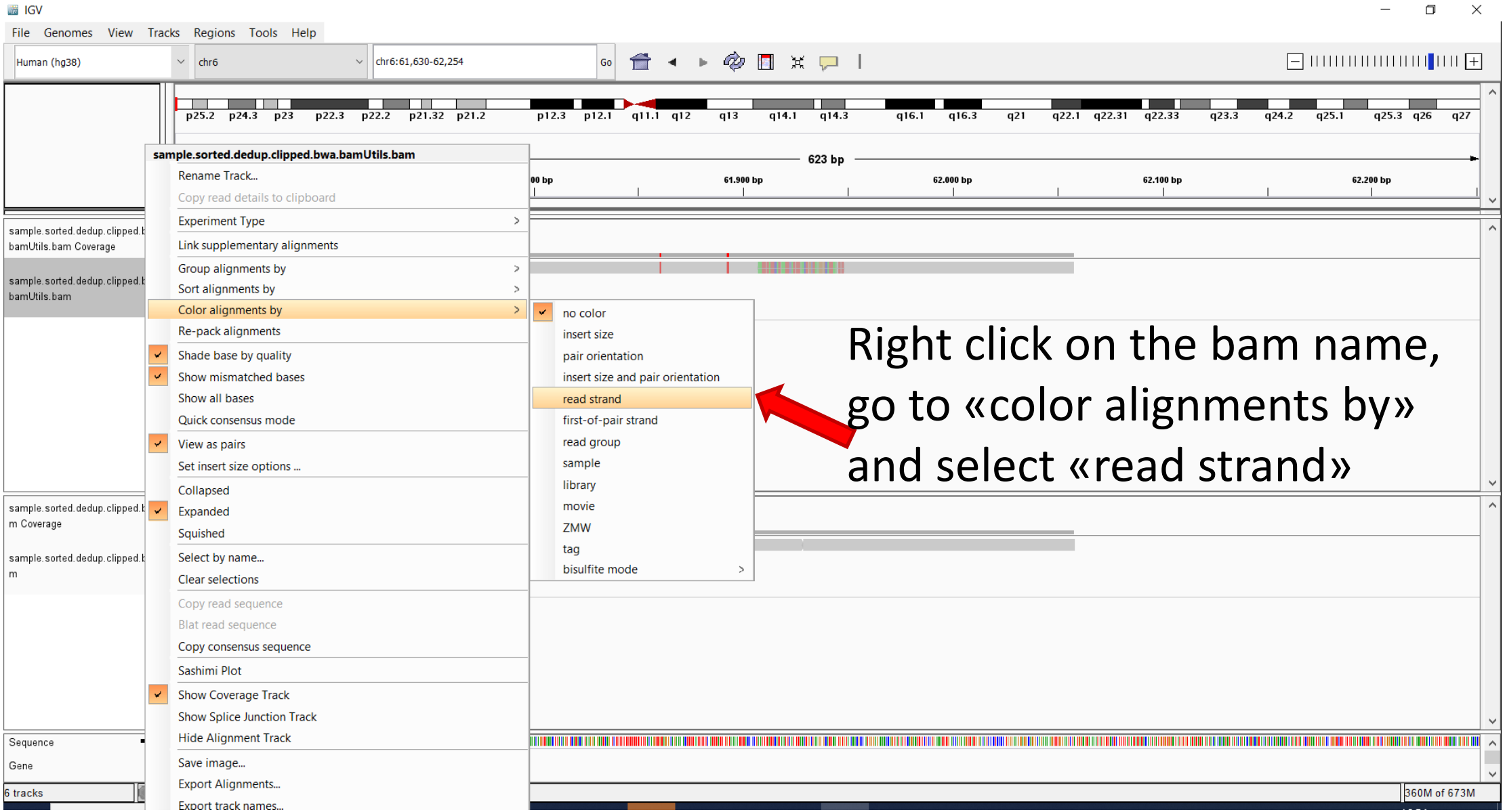



Select «show soft-clipped bases» and save

# Check differences between soft and hard clipping



# Check differences between soft and hard clipping




Right click on the bam name, go to «color alignments by» and select «read strand»

# Results differences between soft and hard clipping