Genomic Computing

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1 Setting

After login and qlogin there was not a public_html directory. I went on creating a homework directory and linking the data files in there.

```
smosciat@dpt-n1:~$ mkdir homework
smosciat@dpt-n1:~$ cd homework/
smosciat@dpt-n1:~/homework$ ln -sn /home/lriva/lesson/homeworkFastqfiles/gata1
smosciat@dpt-n1:~/homework$ ln -sn /home/lriva/lesson/homeworkFastqfiles/tal1
smosciat@dpt-n1:~/homework$ ln -sn /home/lriva/lesson/homeworkFastqfiles/input
smosciat@dpt-n1:~/homework$ ln -sn /home/lriva/public_html/lessonpolimi/chrom
smosciat@dpt-n1:~/homework$ ls
```

2 Part 1

We will start looking for the quality of our sample.

```
$ fastx_quality_stats -Q33 -i tal1.fastq -o tal1_quality.txt
$ fastx_quality_stats -Q33 -i gata1.fastq -o gata1_quality.txt
$ fastx_quality_stats -Q33 -i input.fastq  -o input_quality.txt
$ ls
chromsizes.tab  gata1.fastq  gata1_quality.txt  input.fastq  input_quality.tx
tal1.fastq  tal1_quality.txt
```

Then we explore the output files which any of the unix tools we have at our disposal, I like less.

```
# printing the minimun, the mean, the first quartile,
# the median, the third quartile and the Inter Quartile Range
smosciat@dpt-n1:~/homework$ less gata1_quality.txt | awk {'print $3, $6, $7, $
2 34.87 33 38 39 6
2 34.78 33 38 39 6
2 34.67 33 38 39 6
2 34.52 33 38 39 6
2 34.30 33 38 39 6
```

```
2 34.34 33 38 39 6
2 34.38 33 38 39 6
2 34.33 33 38 39 6
2 34.27 33 38 39 6
2 34.36 34 38 39 5
smosciat@dpt-n1:~/homework$ less tal1_quality.txt | awk {'print $3, $6, $7, $8
2 35.36 35 38 40 5
2 35.26 35 38 40 5
2 35.15 35 38 40 5
2 35.00 34 38 39 5
2 34.76 34 38 39 5
2 34.81 34 38 40 6
2 34.83 34 38 40 6
2 34.80 34 38 40 6
2 34.76 34 38 40 6
2 34.85 35 38 40 5
smosciat@dpt-n1:~/homework$ less input_quality.txt | awk {'print $3, $6, $7, $
2 35.00 34 38 39 5
2 34.86 33 38 39 6
2 34.82 33 38 39 6
2 34.65 33 38 39 6
2 34.56 33 38 39 6
2 34.55 33 38 39 6
2 34.49 33 38 39 6
2 34.47 33 38 39 6
2 34.43 33 38 39 6
2 34.53 35 38 39 4
```

Even at the bottom of the file we can see a very high quality score for this files, even the first quartile is quite close to 40.

The sequences lenght is 41 for all three samples, we can obtain it with:

```
$ wc -l tal1_quality.txt gata1_quality.txt input_quality.txt
   42 tal1_quality.txt
   42 gata1_quality.txt
   42 input_quality.txt
  126 total
# 41 for the sequence plus one for the header
```

Similarly we could have obtained similar information running '*fastqc*' on those same files.

Also, while the quality is overall pretty good, we will see that the minimun is extremelly low, 2, this will be fixed by the filtering procedure in the next steps.

3 Part 2

We pipe everything together to avoid to generate intermediate files.

```
$ fastx_artifacts_filter -i input.fastq -Q33 | \
    fastq_quality_trimmer -t 20 -l 30 -Q33 | \
    fastq_quality_filter -q 20 -p 80 -Q33 > input_filtered.fastq
$ fastx_artifacts_filter -i gata1.fastq -Q33 | \
    fastq_quality_trimmer -t 20 -l 30 -Q33 | \
    fastq_quality_filter -q 20 -p 80 -Q33 > gata1_filtered.fastq
$ fastx_artifacts_filter -i tal1.fastq -Q33 | \
    fastq_quality_trimmer -t 20 -l 30 -Q33 | \
    fastq_quality_filter -q 20 -p 80 -Q33 > tal1_filtered.fastq
$ ls | grep filtered
gata1_filtered.fastq
input_filtered.fastq
tal1_filtered.fastq
```

4 Part 3

At this point we can execute the same analysis of before, we are expecting to see quality values not to different since the quality was already very high, but we should not see very low values in the minimun.

Also we are expecting less sequences.

```
$ head -n 40 gata1_filtered_fastqc/fastqc_data.txt
##FastQC        0.10.0
>>Basic Statistics      pass
#Measure        Value
Filename        gata1_filtered.fastq
File type       Conventional base calls
Encoding        Sanger / Illumina 1.9
Total Sequences 9597230
Filtered Sequences      0
Sequence length 30-41
%GC     43
>>END_MODULE
>>Per base sequence quality      pass
#Base   Mean    Median  Lower Quartile  Upper Quartile  10th Percentile 90th
1       37.037077365031365      38.0    36.0    40.0    33.0    40.0
2       36.69769673124433       38.0    35.0    40.0    31.0    40.0
3       36.74423766024155       38.0    35.0    40.0    31.0    40.0
4       36.684463225326475      38.0    35.0    40.0    31.0    40.0
5       36.74516949161372       38.0    36.0    40.0    31.0    40.0
```

```
6       36.823475315273264      38.0    36.0    40.0    31.0    40.0
7       36.80927069581536       38.0    35.0    40.0    31.0    40.0
8       36.69087705515029       38.0    35.0    40.0    31.0    40.0
9       36.70194181029318       38.0    35.0    40.0    31.0    40.0
10      36.725207273348666      38.0    35.0    40.0    31.0    40.0
11      36.797033414849906      38.0    35.0    40.0    31.0    40.0
12      36.76370213071897       38.0    35.0    40.0    31.0    40.0
13      36.65678388451668       38.0    35.0    40.0    31.0    40.0
14      36.699702309937344      38.0    35.0    40.0    31.0    40.0
15      36.651681057971935      38.0    35.0    40.0    31.0    40.0
16      36.70446149566073       38.0    35.0    40.0    31.0    40.0
17      36.676404024911356      38.0    35.0    40.0    31.0    40.0
18      36.54339033241883       38.0    35.0    40.0    30.0    40.0
19      36.583383226201725      38.0    35.0    40.0    31.0    40.0
20      36.50235213702287       38.0    35.0    40.0    30.0    40.0
21      36.55767893444254       38.0    35.0    40.0    31.0    40.0
22      36.46454539486914       38.0    35.0    40.0    30.0    40.0
23      36.383993506459674      38.0    35.0    40.0    30.0    40.0
24      36.32412571127294       38.0    35.0    40.0    30.0    40.0
25      36.35646618868152       38.0    35.0    40.0    30.0    40.0
26      36.36381320443503       38.0    35.0    40.0    30.0    40.0
27      36.16238518822619       38.0    35.0    40.0    30.0    40.0
$
$ head -n 40 tal1_filtered_fastqc/fastqc_data.txt
##FastQC       0.10.0
>>Basic Statistics      pass
#Measure        Value
Filename        tal1_filtered.fastq
File type       Conventional base calls
Encoding        Sanger / Illumina 1.9
Total Sequences 9668892
Filtered Sequences      0
Sequence length 30-41
%GC     44
>>END_MODULE
>>Per base sequence quality      pass
#Base   Mean    Median  Lower Quartile  Upper Quartile  10th Percentile 90th
1       37.358686807133644      39.0    36.0    40.0    33.0    40.0
2       37.04603774662081       39.0    36.0    40.0    31.0    40.0
3       37.07896716604136       39.0    36.0    40.0    32.0    40.0
4       37.03074188852249       39.0    36.0    40.0    31.0    40.0
5       37.08440770669483       39.0    36.0    40.0    32.0    40.0
6       37.15360984485089       39.0    36.0    40.0    33.0    40.0
```

```
7       37.134781110389895      39.0    36.0    40.0    32.0    40.0
8       37.02007706777571       39.0    36.0    40.0    31.0    40.0
9       37.04320308883376       39.0    36.0    40.0    31.0    40.0
10      37.037372948213715      39.0    36.0    40.0    31.0    40.0
11      37.118752593368505      39.0    36.0    40.0    32.0    40.0
12      37.06046918302531       39.0    36.0    40.0    32.0    40.0
13      36.99266286147368       39.0    36.0    40.0    31.0    40.0
14      37.01429905308695       39.0    36.0    40.0    31.0    40.0
15      36.97077586552834       39.0    36.0    40.0    31.0    40.0
16      37.038785209308365      39.0    36.0    40.0    32.0    40.0
17      36.99016133389431       39.0    36.0    40.0    31.0    40.0
18      36.86733722953985       38.0    36.0    40.0    31.0    40.0
19      36.90105619134023       38.0    36.0    40.0    31.0    40.0
20      36.825560674377165      38.0    35.0    40.0    31.0    40.0
21      36.88942548949766       38.0    36.0    40.0    31.0    40.0
22      36.879703693039495      38.0    36.0    40.0    31.0    40.0
23      36.812378502107585      38.0    35.0    40.0    31.0    40.0
24      36.73391325500378       38.0    35.0    40.0    31.0    40.0
25      36.768105900862274      38.0    35.0    40.0    31.0    40.0
26      36.69124145765616       38.0    35.0    40.0    31.0    40.0
27      36.5639103218859        38.0    35.0    40.0    31.0    40.0
$
$ head -n 40 input_filtered_fastqc/fastqc_data.txt
##FastQC       0.10.0
>>Basic Statistics      pass
#Measure        Value
Filename        input_filtered.fastq
File type       Conventional base calls
Encoding        Sanger / Illumina 1.9
Total Sequences 9609450
Filtered Sequences      0
Sequence length 30-41
%GC     42
>>END_MODULE
>>Per base sequence quality      pass
#Base   Mean    Median  Lower Quartile  Upper Quartile  10th Percentile 90th
1       37.077514009646755      38.0    36.0    40.0    33.0    40.0
2       36.736964446456355      38.0    35.0    40.0    31.0    40.0
3       36.7529384095864        38.0    36.0    40.0    31.0    40.0
4       36.68676396672026       38.0    35.0    40.0    31.0    40.0
5       36.76905951953546       38.0    36.0    40.0    31.0    40.0
6       36.808094219752434      38.0    36.0    40.0    31.0    40.0
7       36.831154540582446      38.0    36.0    40.0    31.0    40.0
```

8	36.69810530259276	38.0	35.0	40.0	31.0	40.0
9	36.70641816128915	38.0	35.0	40.0	31.0	40.0
10	36.735112519447 38.0	35.0	40.0	31.0	40.0	
11	36.75945439125028	38.0	35.0	40.0	31.0	40.0
12	36.72983219643164	38.0	35.0	40.0	31.0	40.0
13	36.59003813953972	38.0	35.0	40.0	31.0	40.0
14	36.620014881184666	38.0	35.0	40.0	31.0	40.0
15	36.60444447913252	38.0	35.0	40.0	31.0	40.0
16	36.08316480131537	38.0	35.0	40.0	29.0	40.0
17	36.522020094802514	38.0	35.0	40.0	30.0	40.0
18	36.436214351497746	38.0	35.0	40.0	30.0	40.0
19	36.41653060268798	38.0	35.0	40.0	30.0	40.0
20	36.413492343474395	38.0	35.0	40.0	30.0	40.0
21	36.509409071278796	38.0	35.0	40.0	30.0	40.0
22	36.44318571822529	38.0	35.0	40.0	30.0	40.0
23	36.44546014600211	38.0	35.0	40.0	30.0	40.0
24	36.338072938617714	38.0	35.0	40.0	30.0	40.0
25	36.29482374121308	38.0	35.0	40.0	30.0	40.0
26	36.39342168386328	38.0	35.0	40.0	30.0	40.0
27	36.26720738439765	38.0	35.0	40.0	30.0	40.0

The results are close to our expectation.

5 Part 4: Alligment

We start alligning our sequences with the references genome.

```
$ bwa aln -t 4 -f input.sai /db/bwa/0.6.2/mm9/mm9 input_filtered.fastq
$ bwa aln -t 4 -f tal1.sai /db/bwa/0.6.2/mm9/mm9 tal1_filtered.fastq
$ bwa aln -t 4 -f gata1.sai /db/bwa/0.6.2/mm9/mm9 gata1_filtered.fastq
```

Then we move on in creating the indexes.

```
$ bwa samse /db/bwa/0.6.2/mm9/mm9 input.sai input_filtered.fastq | \
    samtools view -ut /db/bwa/0.6.2/mm9/mm9 - | \
    samtools sort - input
$ samtools index input.bam
$
$ bwa samse /db/bwa/0.6.2/mm9/mm9 tal1.sai tal1_filtered.fastq | \
    samtools view -ut /db/bwa/0.6.2/mm9/mm9 - | \
    samtools sort - tal1
$ samtools index tal1.bam
$
$ bwa samse /db/bwa/0.6.2/mm9/mm9 gata1.sai gata1_filtered.fastq | \
    samtools view -ut /db/bwa/0.6.2/mm9/mm9 - | \
```

```
    samtools sort - gata1
$ samtools index gata1.bam
$
```

Now we can visualize how many sequence match, with and without the "-F 4"

```
$ samtools view -h -c -o input.sam input.bam
9609450
$ samtools view -h -F 4 -c -o input.sam input.bam
9364936
$
$ samtools view -h -c -o tal1.sam tal1.bam
9668892
$ samtools view -h -F 4 -c -o tal1.sam tal1.bam
9338125
$
$ samtools view -h -c -o gata1.sam gata1.bam
9597230
$ samtools view -h -F 4 -c -o gata1.sam gata1.bam
8659331
$
```

6 Part 5: Peak Calling

Here we are assuming that the file "input.fastq" contained the control experiment.

The two command used are:

```
$ macs14 -t gata1.bam -c input.bam --name gata1_peakcalling \
    --mfold=10,30 --format=BAM --pvalue=1e-8 -s 41 -g mm --diag
$ macs14 -t tal1.bam -c input.bam --name tal1_peakcalling \
    --mfold=10,30 --format=BAM --pvalue=1e-8 -s 41 -g mm --diag
```

The results are:

```
$ wc -l gata1_peakcalling_peaks.bed \
    gata1_peakcalling_negative_peaks.xls \
    tal1_peakcalling_peaks.bed \
    tal1_peakcalling_negative_peaks.xls

  2660 gata1_peakcalling_peaks.bed
     9 gata1_peakcalling_negative_peaks.xls
  1438 tal1_peakcalling_peaks.bed
    12 tal1_peakcalling_negative_peaks.xls
  4119 total
```

So we have called 2660 peaks for the gata1 and 8 of them are negative, and 1438 for the tal1 and 11 of them are negative.

(The negative files contains a line of header.)

```
$ cat gata1_peakcalling_diag.xls
FC range        # of Peaks       cover by sampling 90%   80%      70%
0-20            968                                53.20   42.25    33.06
20-40           1256                               90.76   84.32    77.15
40-60           308                                100.00  100.00   100.00
$
$ cat tal1_peakcalling_diag.xls
FC range        # of Peaks       cover by sampling 90%   80%      70%
0-20            244                                77.05   62.70    57.79
20-40           752                                93.48   85.51    79.92
40-60           296                                100.00  100.00   99.66
```

The saturation level at the enrichment between 20 and 40 with 90% of the fields are 90.76 and 93.48, respectively for gata1 and tal1.

7 Part 6: Shared Peaks

```
$ intersectBed -a tal1_peakcalling_peaks.bed \
    -b gata1_peakcalling_peaks.bed -u > intersect_peaks.bed
$
$ wc -l intersect_peaks.bed
1148 intersect_peaks.bed
```

I interpreted the request of the point 6.2 as to find the peaks in gata1 that are not in tal1.

```
$ intersectBed -b tal1_peakcalling_peaks.bed \
    -a gata1_peakcalling_peaks.bed -v > peaks_only_in_gata1.bed
$
$ wc -l peaks_only_in_gata1.bed
1514 peaks_only_in_gata1.bed
```

8 Part 7

Here we create the bw files.

```
$ bamToBed -i gata1.bam | \
    slopBed -i stdin -g chromsizes.tab -s -r 160 -l 0  | \
    genomeCoverageBed -i stdin -g chromsizes.tab -bg > tmp.wig; \
    wigToBigWig tmp.wig chromsizes.tab gata1.bw; \
    rm tmp.wig
$ bamToBed -i tal1.bam | \
    slopBed -i stdin -g chromsizes.tab -s -r 160 -l 0  | \
    genomeCoverageBed -i stdin -g chromsizes.tab -bg > tmp.wig; \
```

```
    wigToBigWig tmp.wig chromsizes.tab tal1.bw; \
    rm tmp.wig
$ bamToBed -i input.bam | \
    slopBed -i stdin -g chromsizes.tab -s -r 160 -l 0   | \
    genomeCoverageBed -i stdin -g chromsizes.tab -bg > tmp.wig; \
    wigToBigWig tmp.wig chromsizes.tab input.bw; \
    rm tmp.wig
```

9 Part 8

```
$ cut -f 1-3 intersect_peaks.bed > intersect_GREAT.bed
$ cut -f 1-3 peaks_only_in_gata1.bed > peak_only_in_gata1_GREAT.bed
```

The relative motif are:

Figure 1: Motif for peaks in common

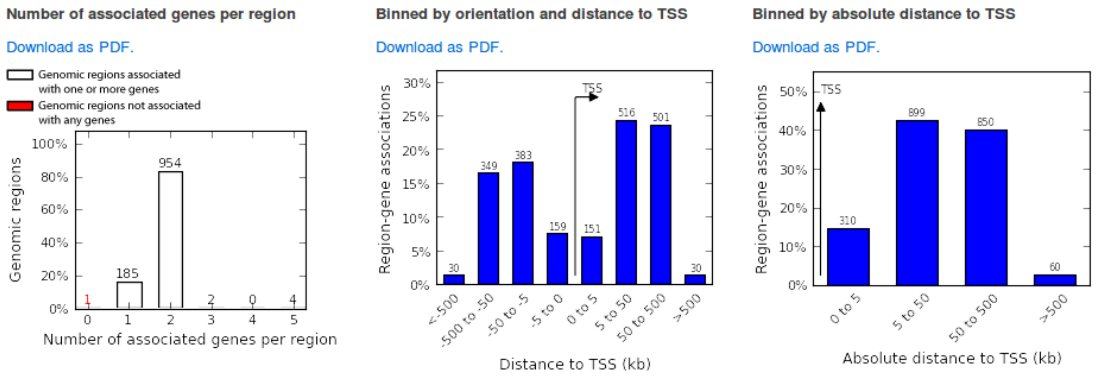

Figure 2: Motif for peaks of only GATA1