MCB 5430 midterm assignment

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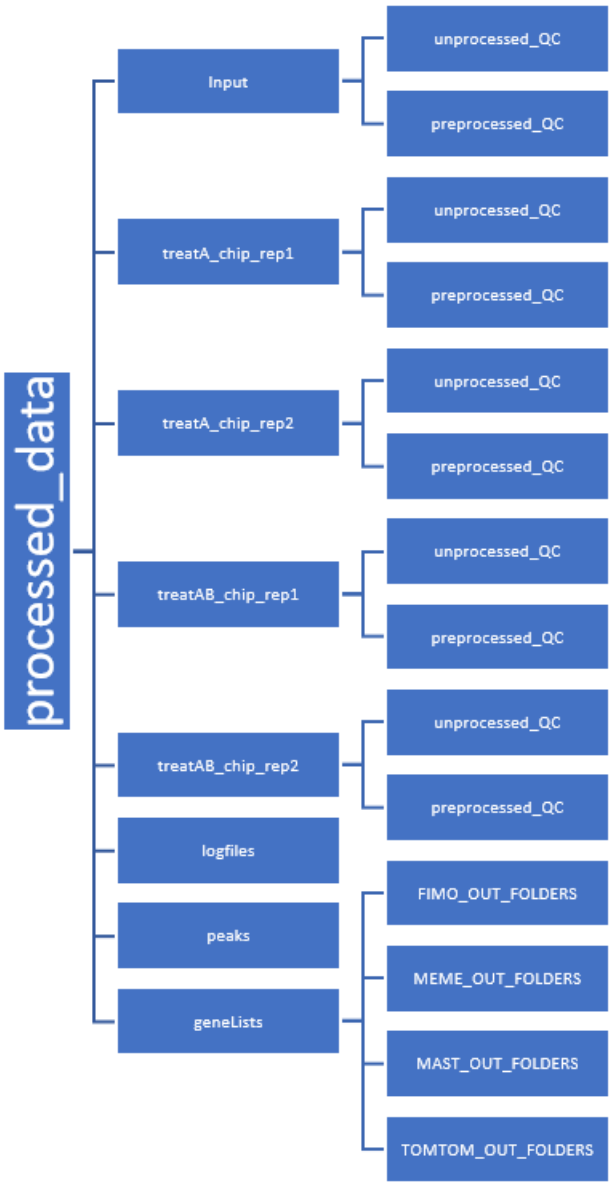


Tree folder structure of the script output

1 ChIP-seq processing, alignment and display

1.1 Pipeline

The shell script used in processing all of the data in this midterm can be found here.

1.2 Summary table

The mapping of the reads has been done with the -m 1 option. Therefore, the "reads with at least one reported alignment" field in the log file refers to the number of uniquely mapped reads that fulfilled the mapping parameters. Table 1 displays the summary of the reads alignment step of the pipeline.

Table 1. Summary table for the alignment step of the pipeline. Reads total refers to the number of fastq reads that passed the preprocessing steps of the pipeline. "# and % unique" display the number and the percentage of uniquely mapped reads. "# and % multiple" display the number and the percentage of multiply mapped reads that got discarded due to the constrains of the mapping parameter -m 1. "# and % unaligned" display the number and percentage of reads that did not align to the hg19 genome.

Sample	# reads total	# unique	# multiple	# unaligned	% unique	% multiple	% unaligned
Input	12,265,901	10,088,192	1,016,900	1,160,809	82.25	8.29	9.46
treatment A rep 1	13,164,770	10,716,882	921,317	1,526,571	81.41	7.00	11.60
treatment A rep 2	14,071,793	11,248,659	971,196	1,851,938	79.94	6.90	13.16
treatment AB rep 1	13,132,269	10,686,406	955,345	1,490,518	81.38	7.27	11.35
treatment AB rep 2	8,725,137	6,948,241	601,088	1,175,808	79.63	6.89	13.48

2 Genome browser shot

The genome browser PDF document displays the highest peak on Chromsome 12 and can be found here.

3 Peak calling and analysis

The following part of the script includes the peak calling step, high confidence peak files generation and getting the peaks unique to each treatment. Since they were all typed in a single for loop, they are displayed together below (therefore the for loop ends on next page).

3.1 MACS

```
158
159
160 #=================================================================================================================
    ================================================
161 # This part is for calling peaks using MACS. After peak calling, it shifts the peaks by half the "d" value
    that the pdf reports and creates files for genome
162 # browser use by adding the BED headers. The genome browser will display a region 300 nts upstream and
    downstream of the top peak found in chromosome 12.
163 #For MEME and FIMO usage, one should use the top summits from the entire set of chromosomes (w/ file
    provided on the server)
164 #=================================================================================================================
    ================================================
165     cd ..
166     echo "Calling peaks for Chromosome 12 using MACS"
167
168     if [ -s ${outPATH}peaks ]
169     then
170         cd peaks
171     else
172         mkdir peaks
173         cd peaks
174     fi
175
176 for file in $fastqfiles
177     do
178         ext=`echo $(basename $file) | cut -d "." -f 2` # generated to see file type
179         prefix=`echo $(basename $file) | cut -d "." -f 1`  #creates a prefix for each fastq file that is
            analyzed
180
181         if [ $prefix != "Input" ]
182         then
183             macs14 -t ${outPATH}${prefix}/${prefix}_chr12.sorted.bam -c ${outPATH}Input/
                Input_chr12.sorted.bam -f BAM -n ${prefix} -g 133851895
184             Rscript ${prefix}_model.r
185             peakshift=`grep "legend" ${prefix}_model.r | tail -n 1 | cut -d "=" -f2 | cut -d "\"" -f1`
186
187             top_peak=`sort -k5nr ${prefix}_summits.bed | head -1 | cut -f2`
188             browser_start=$(($top_peak - 300))
189             browser_end=$(($top_peak + 300))
190
191             echo "Shifting peaks by $peakshift"
192             awk -v d=$peakshift '{printf ("%s\t%s\t%s\t%s\t%s\n", $1, $2 + (d/2), $3 - (d/2), $4, $5)}'
                 ${prefix}_peaks.bed > ${prefix}_peaks_shifted.bed
193
194             echo "Generating UCSC BED files with headers for peaks and summits"
195
196             awk -v NAME=${prefix}_peaks -v browser_start=$browser_start -v browser_end=$browser_end
                'BEGIN { print "browser position chr12:("browser_start")-("browser_end")"
197             print "track type=bed name=\""NAME"\" description=\""NAME"\" visibility=squish
                autoScale=on colorByStrand=\"255,0,0 0,0,255\""}
198             { print $0}' ${prefix}_peaks_shifted.bed > ${prefix}_peaks_shifted_header.bed
199
200             awk -v NAME=${prefix}_summits -v browser_start=$browser_start -v browser_end=$browser_end
                'BEGIN { print "browser position chr12:("browser_start")-("browser_end")"
201             print "track type=bed name=\""NAME"\" description=\""NAME"\" visibility=squish
                autoScale=on colorByStrand=\"255,0,0 0,0,255\""}
202             { print $0}' ${prefix}_summits.bed > ${prefix}_summits_header.bed
203
```

3.2 High confidence peaks and 2.3 Peaks specific to each treatment

```
#=================================================================================================
# This part intersects the datasets to report:
# 1. High confidence peaks between replicates
# 2. Peaks specific only to treatment A or only to treatment A+B
#=================================================================================================

                sample=`echo $prefix | cut -d "_" -f1,2`

                if [ -s ${sample}_rep1_peaks_shifted.bed ] && [ -s ${sample}_rep2_peaks_shifted.bed ]
                then
                    echo "Finding high confidence peaks between replicates"
                    bedtools intersect -a ${sample}_rep1_peaks_shifted.bed -b ${sample}
                    _rep2_peaks_shifted.bed > ${sample}_peaks_highconf.bed

# the next statement is a bit iffy because it needs the other sample high confidence peaks bed file, and
it depends on the order the files are processed, but works

                    if [ $sample=="treatA_chip" ] && [ -s treatAB_chip_peaks_highconf.bed ]
                    then
                        bedtools intersect -v -a ${sample}_peaks_highconf.bed -b
                        treatAB_chip_peaks_highconf.bed > ${sample}_only_peaks.bed
                    elif [ $sample=="treatAB_chip"] && [ -s treatA_chip_peaks_highconf.bed ]
                    then
                        bedtools intersect -v -a ${sample}_peaks_highconf.bed -b
                        treatA_chip_peaks_highconf.bed > ${sample}_only_peaks.bed
                    fi
                fi
            fi


    done | tee -a ${outPATH}logfiles/log.txt


cd $outPATH
```

Observation: The file containing peaks unique to treatment AB only is an empty file. This does not seem to be a script error - upon analysis of the peaks common to both treatments and peaks unique to treatment A, it is likely that AB peaks are a subset of A peaks. This already might mean that whatever treatment AB is, it inhibits the binding of this transcription factor to DNA.

4 Distribution of TF binding sites

4.1 Bed files with promoter, gene and intergenic sequences

For this step, TSS abbreviation of the files stands for the promoters, genes represent the genes and IGS represent the intergenic sequences. In addition to .bed files for each region type, .fasta files have been created here as well.

```
237 echo "Generating gene lists" | tee -a ${outPATH}logfiles/log_geneLists.txt
238 mkdir geneLists
239 cd geneLists
240
241
242 #=============================================================================================================
    ==========
243 # This part processes the hg19_gencode_ENSG_geneID.bed file to retrieve the TSS, promoters, genes and
    intergenic regions for chromosome 12
244 #=============================================================================================================
    ==========
245
246
247 # Retrieving only chromosome 12 entries:
248
249 echo "Retrieving chr12 entries"  | tee -a ${outPATH}logfiles/log_geneLists.txt
250 cat $gencode | grep "chr12" > ./gencode_ENSG_geneID_chr12.txt
251
252 # Retrieving TSS - if the gene is on the + strand, start of gene is $2 => subtract 500 from $2 (start of
    TSS) and add 500 to $2 (end of TSS)
253 # If the gene is on the - strand, the start of the gene is $3 => add 500 to $3 (start of TSS) and subtract
    500 to $3 (end of TSS)
254 # In these BED files, the smaller coordinate is always in col. 2, regardless of the strand
255
256 echo "Creating TSS only file" | tee -a ${outPATH}logfiles/log_geneLists.txt
257 awk '{if($6=="+" && $2<$3)
258 printf ("%s\t%s\t%s\t%s\t%s\t%s\n", $1, $2 - 500, $2 + 500, $4, $5, $6);
259 else if($6=="-" && $2<$3)
260 printf ("%s\t%s\t%s\t%s\t%s\t%s\n", $1, $3 - 500, $3 + 500, $4, $5, $6)
261 }' gencode_ENSG_geneID_chr12.txt > gencode_ENSG_geneID_chr12_TSS.bed
262 bedtools getfasta -name -fi $hg19 -bed gencode_ENSG_geneID_chr12_TSS.bed -fo
    gencode_ENSG_geneID_chr12_TSS.fasta
263
264 # Retrieving genes - if the gene is on the + strand, gene region starts at $2 + 501 and ends at the $3 + 1000
265 # if the gene is on the - strand, the gene ends at $2 - 1000, starts at $3 - 501
266 # In these BED files, the smaller coordinate is always in col. 2 regardless of the strand
267
268 echo "Creating genes only file" | tee -a ${outPATH}logfiles/log_geneLists.txt
269 awk '{if($6=="+" && $2<$3)
270 printf ("%s\t%s\t%s\t%s\t%s\t%s\n", $1, $2 + 501, $3 + 1000, $4, $5, $6);
271 else if($6=="-" && $2<$3)
272 printf ("%s\t%s\t%s\t%s\t%s\t%s\n", $1, $2 - 1000, $3 - 501, $4, $5, $6)
273 }' gencode_ENSG_geneID_chr12.txt > gencode_ENSG_geneID_chr12_genes.bed
274 bedtools getfasta -name -fi $hg19 -bed gencode_ENSG_geneID_chr12_genes.bed -fo
    gencode_ENSG_geneID_chr12_genes.fasta
275
276
277 # Intergenic regions - basically if it's not part of the first two - intersect with chromosome 12 file and
    take the complement
278
279 echo "Creating IGS only file" | tee -a ${outPATH}logfiles/log_geneLists.txt
280 #creating a temporary file with both TSS and genes and creating the chromosome 12 only file size
281
282 grep chr12 $hg19chromInfo > ./chr12Info.txt
283
284 cat ./gencode_ENSG_geneID_chr12_TSS.bed >> gencode_ENSG_geneID_chr12_genesandTSS.bed
285 cat ./gencode_ENSG_geneID_chr12_genes.bed >> gencode_ENSG_geneID_chr12_genesandTSS.bed
286
287 # intersecting the file with chromosome 12
288 bedtools sort -i gencode_ENSG_geneID_chr12_genesandTSS.bed > gencode_ENSG_geneID_chr12_genesandTSS.sorted.bed
289 bedtools complement -i gencode_ENSG_geneID_chr12_genesandTSS.sorted.bed -g chr12Info.txt >
    gencode_ENSG_geneID_chr12_IGS.bed
290 rm gencode_ENSG_geneID_chr12_genesandTSS.*
291 bedtools getfasta -name -fi $hg19 -bed gencode_ENSG_geneID_chr12_IGS.bed -fo
    gencode_ENSG_geneID_chr12_IGS.fasta
292
293
```

4.2 Determining the distribution of high confidence peaks (summits) that fall into promoter, genes and intergenic sequences

This entire code block is also under the same for loop which analyzes everything down to the TOMTOM step (therefore, it's far from the 'done' line)

```
#=================================================================================================================
# This part analyzes the chromosome 12 summits from treatments A and A+B in order to see the distribution of the peaks that fall in the TSS, genes and IGS regions
# The summits files are the ones provided on /tempdata3/MCB5430/midterm/midterm/peaks folder which are the from the entire genome
# Also generates fasta files for MEME motif analysis and finds the MEME motifs
#=================================================================================================================

for file in $summits_highconf
    do
        ext=`echo $(basename $file) | cut -d "." -f 2` # generated to see file type
        prefix=`echo $(basename $file) | cut -d "." -f 1`  #creates a prefix for each bed file that is analyzed
        echo "Starting analysis for high confidence summits for $prefix"
        grep chr12 $file > ${prefix}_chr12.bed

        echo "Examining distribution in TSS, IGS, genes..."
        if [ $ext=="bed" ]
            then
            bedtools coverage -a ${prefix}_chr12.bed -b gencode_ENSG_geneID_chr12_TSS.bed  > ${prefix}_chr12_inTSS.bed
            bedtools coverage -a ${prefix}_chr12.bed -b gencode_ENSG_geneID_chr12_IGS.bed > ${prefix}_chr12_inIGS.bed
            bedtools coverage -a ${prefix}_chr12.bed -b gencode_ENSG_geneID_chr12_genes.bed > ${prefix}_chr12_ingenes.bed

            awk '{if($9!="0.0000000")
            print $0}' ${prefix}_chr12_inTSS.bed > ${prefix}_chr12_inTSS_nozero.bed

            awk '{if($9!="0.0000000")
            print $0}' ${prefix}_chr12_ingenes.bed > ${prefix}_chr12_ingenes_nozero.bed

            awk '{if($9!="0.0000000")
            print $0}' ${prefix}_chr12_inIGS.bed > ${prefix}_chr12_inIGS_nozero.bed

        fi
```

4.3 Summary table for distribution in genomic regions

Table 2 contains a summary for the section 3.2 and for MAST and FIMO. The following example calculations show how the table was compiled:

1. Grey rows: determining the distribution of high confidence peaks (summits) in different genome regions

The numbers for overlapping summits for each genomic region are present in the columns starting with #. The Total represents the sum of all summits falling in promoters, genes and intergenic sequences, and the percentages are calculated by dividing the number for each genomic region by the total number of summits:

% in genes = (# in genes / # Total) * 100

e.g. For Treatment A:
% in genes = (# in genes / # Total) * 100
% in genes = (411 / 1032) * 100
% in genes = **39.82**.

2. MAST outputs: determining how many peaks have motifs (peaks being broken down by different genome regions)

Starting for example with *treatment A intergenic regions*, 117 peaks have motif 1 and 137 peaks have motif 2. The total number of peaks with motifs for treatment A is therefore 117+137 = 254. To obtain the number of peaks without motifs, 254 was subtracted from the *total peaks from intergenic regions* calculated in section 3.2 (in the grey bar):

peaks with no motifs in IGS = total # peaks in IGS - total # peaks w/ motifs in IGS
peaks with no motifs = 603 - 254 = **349**

To further calculate the percentages, I divided the number of peaks (having a certain motif in a certain genomic region) by the total number of peaks, regardless of genomic region. For example, for the treatment A intergenic regions for motif 1, the calculations were as following:

% in IGS = (# in IGS / # Total) * 100
% in IGS = (117 / 1032) * 100
% in IGS = 11.34

This way, by looking at a genomic region percentages column, the white part of the table is essentially a breakdown of the grey row percentage. The table can be read, for example, like this: for treatment A, 58.43% of peaks are in intergenic regions and out of 58.43 percents 11.34 of them have motif 1, 13.28 of them have motif 2 and 33.82 of them display no motif.

Table 2. Summary of MAST, FIMO and distribution of summits in different genomic regions. Certain features determined with either MAST, FIMO or bedtools are broken down by intergenic regions. Raw number of peaks as well as calculated percentages of the total are displayed.

		Feature	# in promoters	% in promoters	# in genes	% in genes	# in IGS	% in IGS	# Total	% peaks with motif
	Treatment A	Total peaks	18	1.74	411	39.82	603	58.43	1032	
MAST		Peaks with motif 1	1	0.10	81	7.85	117	11.34	199	
		Peaks with motif 2	0	0.00	92	8.91	137	13.28	229	41.47
		Peaks with motifs (1+2)	1	0.10	173	16.76	254	24.61	428	
		Peaks with no motifs	17	1.65	238	23.06	349	33.82	604	
	Treatment A+B	Total peaks	6	3.10	79	40.93	108	55.95	193	
MAST		Peaks with motif 1	0	0.00	11	5.70	21	10.88	32	
		Peaks with motif 2	0	0.00	15	7.77	17	8.81	32	33.16
		Peaks with motifs (1+2)	0	0.00	26	13.47	38	19.69	64	
		Peaks with no motifs	6	3.11	53	27.46	70	36.27	129	
FIMO	Treatment A	Total motif occurrences	870	0.95	41395	45.07	49576	53.98	91841	
		Motif 1 in Chr. 12	354	0.39	18637	20.29	22668	24.68	41659	
		Motif 2 in Chr. 12	516	0.56	22758	24.78	26908	29.30	50182	
	Treatment A+B	Total motif occurrences	848	0.90	40666	43.27	52464	55.83	93978	
		Motif 1 in Chr. 12	420	0.45	22033	23.44	27851	29.64	50304	
		Motif 2 in Chr. 12	428	0.46	18633	19.83	24613	26.19	43674	

4.4 TF preferences

This transcription factor's preference for promoters or enhancers can be assessed by looking at the number of peaks falling in promoter regions and outside promoter regions. According to Table 2, for treatment A, 1.74% of the summits are in the promoter regions, while 98.25% are in genes and intergenic sequences. For treatment A+B, 3.10 % of the summits are in the promoter regions, while 96.89% of the summits are in genes and intergenic

sequences. This means that the transcription factor has a strong preference to bind **enhancers**, rather than promoters.

5 Identifying motifs under peaks

5.1 MEME

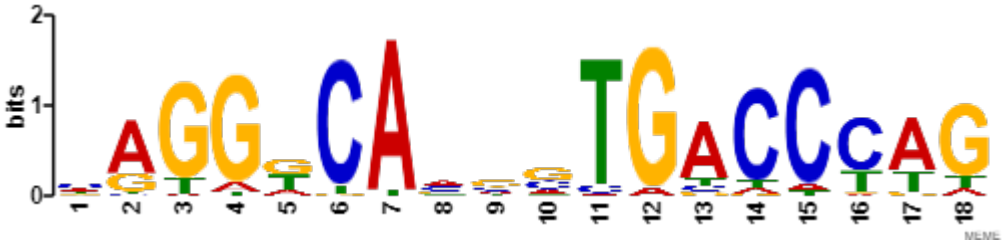
```
323
324         echo "Retrieving top 200 peaks from the entire genome"
325         sort -k5nr $file | head -n 200 > ${prefix}_top200.bed
326         bedtools slop -i ${prefix}_top200.bed -g $hg19chromInfo -b 50 > ${prefix}_top200_100bp.bed
327         bedtools getfasta -name -fi $hg19 -bed ${prefix}_top200_100bp.bed -fo ${prefix}_top200_100bp.fasta
328
329         echo "Finding motifs with MEME for $prefix"
330
331         if [ ${prefix}=="treatA_summits" ]
332             then
333             meme ${prefix}_top200_100bp.fasta -oc ${prefix}_meme_OUT_FOLDER -bfile $TSSbackground -dna -
                nmotifs 2 -minw 10 -maxw 18 -revcomp -mod anr
334             else
335             meme ${prefix}_top200_100bp.fasta -oc ${prefix}_meme_OUT_FOLDER -bfile $TSSbackground -dna -
                nmotifs 2 -minw 12 -maxw 14 -revcomp -mod anr
336         fi
```

Using the code block above, the following motifs have been found (not displaying the reverse complements):

Treatment A

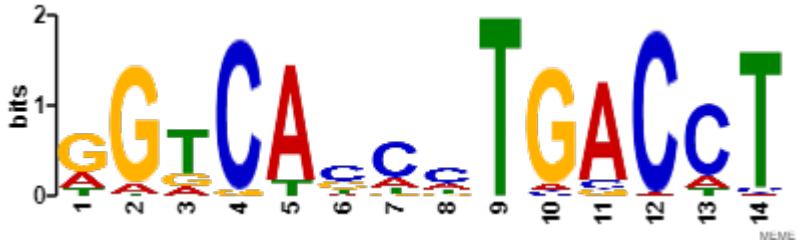
Motif 1:

Motif 2:



Treatment A+B

Motif 1:



Motif 2:

5.2 MAST

```
#=========================================================================================================================
# This part generates fasta sequences for the chromosome 12 TSS, genes and IGS and returns how many of each display the motifs identified with MEME
# (this is done using MAST). It also scans the entire chromosome 12 for motif occurrences (regardless of them being in peaks or not, using FIMO)
#=========================================================================================================================
# The starting files are the three summits files with peaks in TSS, IGS and genes. They need to be expanded 50 bps each way and then converted to multi fasta


        echo "Examining the motif occurrence within chromosome 12 IGS/TSS/genes summits"
        echo "Generating fasta files for each region"
        bedtools slop -i ${prefix}_chr12_inIGS_nozero.bed -g chr12Info.txt -b 50 > ${prefix}_chr12_inIGS_100bp.bed
        bedtools slop -i ${prefix}_chr12_inTSS_nozero.bed -g chr12Info.txt -b 50 > ${prefix}_chr12_inTSS_100bp.bed
        bedtools slop -i ${prefix}_chr12_ingenes_nozero.bed -g chr12Info.txt -b 50 > ${prefix}_chr12_ingenes_100bp.bed

        bedtools getfasta -name -fi $hg19 -bed ${prefix}_chr12_inIGS_100bp.bed -fo ${prefix}_chr12_inIGS_100bp.fasta
        bedtools getfasta -name -fi $hg19 -bed ${prefix}_chr12_inTSS_100bp.bed -fo ${prefix}_chr12_inTSS_100bp.fasta
        bedtools getfasta -name -fi $hg19 -bed ${prefix}_chr12_ingenes_100bp.bed -fo ${prefix}_chr12_ingenes_100bp.fasta

#MAST syntax for each file:
        echo "Searching for MEME motifs in chromosome 12 peaks"
        mast ${prefix}_meme_OUT_FOLDER/meme.txt ${prefix}_chr12_inIGS_100bp.fasta -oc ${prefix}_IGS_mast_OUT_FOLDER
        mast ${prefix}_meme_OUT_FOLDER/meme.txt -hit_list ${prefix}_chr12_inIGS_100bp.fasta -oc ${prefix}_IGS_mast_OUT_FOLDER > ${prefix}_IGS_mast_OUT_FOLDER/list_mast_hits.txt

        mast ${prefix}_meme_OUT_FOLDER/meme.txt ${prefix}_chr12_inTSS_100bp.fasta -oc ${prefix}_TSS_mast_OUT_FOLDER
        mast ${prefix}_meme_OUT_FOLDER/meme.txt -hit_list ${prefix}_chr12_inTSS_100bp.fasta -oc ${prefix}_TSS_mast_OUT_FOLDER > ${prefix}_TSS_mast_OUT_FOLDER/list_mast_hits.txt

        mast ${prefix}_meme_OUT_FOLDER/meme.txt ${prefix}_chr12_ingenes_100bp.fasta -oc ${prefix}_genes_mast_OUT_FOLDER
        mast ${prefix}_meme_OUT_FOLDER/meme.txt -hit_list ${prefix}_chr12_ingenes_100bp.fasta -oc ${prefix}_genes_mast_OUT_FOLDER > ${prefix}_genes_mast_OUT_FOLDER/list_mast_hits.txt
```

5.3 FIMO

```
#FIMO

        echo "Generating chromosome 12 background file for FIMO"
        fasta-get-markov $chr12 > chr12_bkgrnd.txt

        echo "Using FIMO on Chromosome 12 (whole)"
        fimo --oc ${prefix}_Chr12_all_fimo_OUT_FOLDER --bgfile chr12_bkgrnd.txt ${prefix}_meme_OUT_FOLDER/
        meme.txt $chr12
        echo "Using FIMO on Chromosome 12 IGS sequences"
        fimo --oc ${prefix}_IGS_fimo_OUT_FOLDER --bgfile chr12_bkgrnd.txt ${prefix}_meme_OUT_FOLDER/
        meme.txt gencode_ENSG_geneID_chr12_IGS.fasta
        echo "Using FIMO on Chromosome 12 TSS sequences"
        fimo --oc ${prefix}_TSS_fimo_OUT_FOLDER --bgfile chr12_bkgrnd.txt ${prefix}_meme_OUT_FOLDER/
        meme.txt gencode_ENSG_geneID_chr12_TSS.fasta
        echo "Using FIMO on Chromsome 12 gene encoding sequences"
        fimo --oc ${prefix}_genes_fimo_OUT_FOLDER --bgfile chr12_bkgrnd.txt ${prefix}_meme_OUT_FOLDER/
        meme.txt gencode_ENSG_geneID_chr12_genes.fasta
        echo "FIMO analysis done!"

        echo "Reformatting FIMO outputs to .bed"

        awk 'NR>1 {printf("%s\t%s\t%s\t%s\t%s\t%s\n", $2, $3, $4, $9, $7, $5)
        }' ${prefix}_Chr12_all_fimo_OUT_FOLDER/fimo.txt > ${prefix}_Chr12_all_fimo_OUT_FOLDER/fimo_chr12.bed

        awk 'NR>1 {printf("%s\t%s\t%s\t%s\t%s\t%s\n", $2, $3, $4, $9, $7, $5)
        }' ${prefix}_IGS_fimo_OUT_FOLDER/fimo.txt > ${prefix}_IGS_fimo_OUT_FOLDER/fimo_IGS.bed

        awk 'NR>1 {printf("%s\t%s\t%s\t%s\t%s\t%s\n", $2, $3, $4, $9, $7, $5)
        }' ${prefix}_TSS_fimo_OUT_FOLDER/fimo.txt > ${prefix}_TSS_fimo_OUT_FOLDER/fimo_TSS.bed

        awk 'NR>1 {printf("%s\t%s\t%s\t%s\t%s\t%s\n", $2, $3, $4, $9, $7, $5)
        }' ${prefix}_genes_fimo_OUT_FOLDER/fimo.txt > ${prefix}_genes_fimo_OUT_FOLDER/fimo_genes.bed

```

In order to generate Table 2 statistics for FIMO, I used pipes to process the fimo.txt file and to find out out of the two motifs, how many occurrences are for motif 1 and how many are for motif 2, for each treatment. I then checked that the sum of the two is consistent with how many motif occurrences FIMO reports in the html file. E.g.

```
cat fimo.txt | cut -f 1 | grep 1 | wc -l    # for motif 1

cat fimo.txt | cut -f 1 | grep 2 | wc -l # for motif 2
```

Provide an explanation for why not all of your peaks have identified motifs.
As observed in Table 2, only 41.47% of the treatment A peaks display one of the two motifs identified using MEME. Likewise, for treatment A+B, only 33.16% of the peaks have a motif. The reasons why we identify peaks without motif can be the following:
1. According to the chosen MEME options, I looked for the best two motifs only - there could be more than two and the percentages of peaks with a motif might be higher than these numbers.
2. Transcription factors, before any sequence specificity, have DNA binding domains, which causes them to be bound to DNA / chromatin even when not active.
3. Many transcription factors are ligand-dependent, which is a great way to finely modulate the regulation of a set of genes in a ligand concentration dependent manner. Different concentrations of ligands can result in various conformational changes for the transcription factors which can bind different DNA sequences in return.
What do the results tell about the likelihood of the TF finding its motif?
According to the FIMO outcomes (Table 2), there is an overwhelming amount of motif sequences the transcription factor could bind on Chromosome 12. By examining the percentages, for example in treatment A+B, it also appears that the proportion of peaks

found in e.g. intergenic sequences (55.95%) is about the same as the proportion of motifs found in intergenic sequences in the genome (53.98 and 55.83% of motifs found on Chromosome 12 are in intergenic sequences).
This is obviously different from a situation where there would be very few motifs in the genome and the transcription factor would find them against all odds. This transcription factor seems to be *statistically favored to encounter its motifs*, but the abundance of motifs makes it *unlikely that the transcription factor finds its way to perform its function, e.g. upregulate a certain gene by binding to a particular enhancer*. We indeed observe it does not bind very many of these motifs, despite their abundance.
The reasons are plenty - it could be a matter of DNA accessibility (perhaps these motifs are tightly bound by histones which have repressive marks, especially in intergenic sequences), or it could be that the transcription factor generally lies at the end of a signaling cascade and its binding to the targets depends on other effector molecules as well, and not on the DNA sequence alone. This adds another degree of complexity which FIMO cannot account for.

5.4 TomTom

```
394     echo "Looking in JASPAR MEME databases (tomtom)"
395     tomtom -eps -m 1 -o ${prefix}_tomtom_OUT ${prefix}_meme_OUT_FOLDER/meme.txt $jaspar_meme
396
```

According to the TomTom search, for treatment A, there are the following candidate transcription factors:

```
       Name MA0258.1
Alt. Name ESR2
Database jaspar.meme
p-value 5.63649e-09
```

```
       Name MA0112.1
Alt. Name ESR1
Database jaspar.meme
p-value 8.99539e-09
```

For treatment A+B, there are the following transcription factors:

```
       Name MA0112.2
Alt. Name ESR1
Database jaspar.meme
p-value 3.00279e-10
```

```
       Name MA0258.1
Alt. Name ESR2
Database jaspar.meme
p-value 2.0445e-08
```

```
       Name MA0066.1
Alt. Name PPARG
Database jaspar.meme
p-value 2.46812e-07
```

6 Final Question: What factor did Billy ChIP, and what were each of the treatments?

From the TomTom hits above, one can confidently say that the antibody Billy did was against the Estrogen Receptor protein ESR1 or ESR2. According to the uniprot database, the following are known about the Estrogen Receptor [3] :

ESR1 binds DNA as a homodimer and it can form heterodimers with ESR2 as well. Binding is followed by a phosphorylation event on both of the monomer subunits.

Generally, ESR is stabilized by phosphorylation (and protected from proteosomal degradation). ESR activity is modulated by signaling pathway kinases that phosphorylate ESR1 as well as its interacting partners.

ESR has three domains - a modulating N-terminal domain, a DNA binding domain (2 zinc fingers) and a C-terminal ligand binding domain. The N-terminal domain can transactivate in a ligand independent manner, while the C-terminal can transactivate in a ligand dependent manner. Transcription is canonically activated through the C-terminal domain by binding of estrogen. As a result of ligand binding, ESR1 associates with a network of coactivators and binds to estrogen responsive elements.

Various mutations of ESR C-terminal domain are associated with disease. Several mutations result in estrogen resistance disease, where the variants have greatly reduced canonical activity in the presence of elevated estrogen levels. The non-classical activity of the mutants (through the estrogen independent domain) is greatly enhanced and this promotes tumor development and progression.[1]

As seen in Table 2, the total number of peaks for treatment A is 1032, while the number of peaks for treatment A+B is 193. An observation described in section 2.3 shows that the peaks in treatment A+B are a subset of treatment A, which means that the addition of B reagent results in a decreased binding of ESR 1 to the responsive elements. All things considered, for Chromsome 12 peaks, this is a 81.2% decrease in binding.

Assuming that the cell lines Billy has used have the wild type estrogen receptor, treatment A could be estrogen itself, while B can be anything that inhibits ESR1.

One popular treatment for breast cancer cells with wild type ESR1 is an aromatase inhibitor. However, this is not a good choice because aromatase uses body levels of androgen hormones as a substrate and only the production of estrogen in a body context would be lowered.

Another candidate for treatment B is tamoxifen, which is a competitor of estrogen in binding to ESR1 and an antagonist. However, tamoxifen binding to ESR1 still results in binding to DNA and repression of transcription, which in a ChIP-seq experiment would not result in differential binding of ESR1 to DNA - the peaks would still come up[2].

It is safe to assume interfering with phosphorylation will result in destabilizing the ESR1 homodimers or ESR1/2 heterodimers that can bind to DNA. Treatment A could be estrogen, while treatment B could be a kinase inhibitor which would show that even in the presence of estrogen, ESR1 cannot bind to the responsive elements. In addition, repressing phosphorylation in general affects the entire signaling pathway and prevents ESR1 interaction with DNA via both ligand dependent and independent domains.

REFERENCES

[1] Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R (2015) *ESR1 mutations as a mechanism for acquired endocrine resistance in breast cancer* Nat Rev Clin Oncol. 12(10): 573–583.

[2] Wang DY, Fulthorpe R, Liss SN, Edwards EA (2004). *Identification of Estrogen-Responsive Genes by Complementary Deoxyribonucleic Acid Microarray and Characterization of a Novel Early Estrogen-Induced Gene: EEIG1* Molecular Endocrinology, Volume 18, Issue 2, Pages 402–411.

Internet resources:
[3] www.uniprot.org/uniprot/P03372 (Retrieved on Nov. 5, 2017)