ATACseq_Tutorial3_Downstream

September 10, 2023

1 ATAC-seq Module3: Downstream Analysis

1.1 Overview & Purpose

In the previous sections of this module we performed preprocessing quality control, mapping, deduplication, visualization, profiling aroud TSSs, and peak identification. In this section we will focus on differential peak identification, motif footprinting, and annotation of nearby genomic features.

1.1.1 Required Files

In this stage of the module you will use several of the files that we prepared in the previous sections. Don't worry if you are just jumping in now, we have examples of these files saved and will include a step that copies them for your use. You can also use this module on your own data or any published ATAC-seq dataset, but you should complete the mappping and deduplication steps first.

STEP1: Setup Environment

Initial items to configure your google cloud environment. In this step we will use conda to install the following packages:

Differential Peak Idnetification: manorm

Genome Annotation: homer

Motif Analysis: tobias

[1]:
```
#!python -m ipykernel install --user --name ATACtraining
numthreads=!lscpu | grep '^CPU(s)'| awk '{print $2-1}'
numthreadsint = int(numthreads[0])
!conda config --prepend channels bioconda
!conda install -y -c bioconda manorm tobias homer
!pip install jupyterquiz
from jupyterquiz import display_quiz
from IPython.display import IFrame
from IPython.display import display
import pandas as pd
```

```
Warning: 'bioconda' already in 'channels' list, moving to the top
Retrieving notices: …working… done
Collecting package metadata (current_repodata.json): done
Solving environment: unsuccessful initial attempt using frozen solve. Retrying
with flexible solve.
```

```
Solving environment: unsuccessful attempt using repodata from
current_repodata.json, retrying with next repodata source.
Collecting package metadata (repodata.json): done
Solving environment: / ^C
unsuccessful initial attempt using frozen solve. Retrying with flexible solve.

CondaError: KeyboardInterrupt

Requirement already satisfied: jupyterquiz in /opt/conda/lib/python3.10/site-
packages (2.6.3)
```

[6]:
```
# !conda install -y -c bioconda manorm #installed
# !conda install -y -c bioconda tobias ### this is problematic --- build from␣
 ↪source
# !conda install -y -c bioconda homer #installed
```

[2]:
```
# !conda install -y -c bioconda manorm tobias homer
```

```
Collecting package metadata (current_repodata.json): | ^C
/
```

1.2 Setup FileSystem

Now lets create some folders to stay organized and copy over our prepared fastq files. We're going to create a directory called "Tutorial1" which we'll use for this module. We'll then create subfolders for our InputFiles and for the files that we'll be creating during this module. We'll also copy over the fasta file for chromosome 4 as well as some bowtie2 index files (don't worry we'll teach you how to create these index files).

[7]:
```
#These commands create our directory structure.
#!cd $HOMEDIR
#!mkdir -p Tutorial3
#!mkdir -p Tutorial3/InputFiles
!mkdir -p Tutorial3/GenomeAnnotation
!mkdir -p Tutorial3/DiffPeaks
!mkdir -p Tutorial3/MotifFootprinting
#!mkdir -p Tutorial3/LessonImages
!mkdir -p Tutorial3/Plots
#!cd ./Tutorial3
#!echo $PWD

# #These commands help identify the google cloud storage bucket where the␣
 ↪example files are held.
# project_id = "nosi-unmc-seq"
# original_bucket = "gs://unmc_atac_data_examples/Tutorial3"
# #!gsutil -m cp $original_bucket/images/* Tutorial3/LessonImages
# #!gsutil -m cp $original_bucket/Annotations/* Tutorial3/GenomeAnnotations
# #This command copies our example files to the Tutorial1/Inputfiles folder that we created above.
# ! gsutil -m cp $original_bucket/InputFiles/* Tutorial3/InputFiles
```

1.2.1 OK

Let's make sure that the files copied correctly. You should see 2 .bam files, 2 .bai files, and 2 .narrowPeak files after running the following command:

[8]:
```
!ls Tutorial3/InputFiles/*
```

```
Tutorial3/InputFiles/CTL_dedup.bam
Tutorial3/InputFiles/CTL_dedup.bam.bai
Tutorial3/InputFiles/CTL_peaks.narrowPeak
Tutorial3/InputFiles/Mutant_dedup.bam
Tutorial3/InputFiles/Mutant_dedup.bam.bai
Tutorial3/InputFiles/Mutant_peaks.narrowPeak
Tutorial3/InputFiles/chr4.fa
Tutorial3/InputFiles/chr4.fa.fai
```

Differential Peak Identification

If you have two or more samples and desire to discover differential peaks, we recommend using manorm. Novices may be tempted to simply intersect the two peak lists to find the overlap, however this is highly inadvisable.

Interactive Quiz Question 1: Click on the correct answer in following cell.

[9]:
```
display_quiz("Tutorial3/LessonImages/DiffPeaks.json")
```

```
<IPython.core.display.HTML object>
```

```
<IPython.core.display.Javascript object>
```

1.2.2 Consider the below peak which was identified in both the control and mutant sample. A simple intersect would result in this peak being reported as unchanged between the two samples. To represent the differences we will use manorm.

[10]:
```
#We specify several non-default parameters to better reflect ATAC-seq data
!manorm --p1 Tutorial3/InputFiles/CTL_peaks.narrowPeak --p2 Tutorial3/InputFiles/Mutant_peaks.narrowPeak --r1 Tutorial3/InputFiles/CTL_dedup.bam --r2 Tutorial3/InputFiles/Mutant_dedup.bam --rf bam --n1 CTL --n2 Mutant --pe -w 1000 -o Tutorial3/DiffPeaks --wa 2> Tutorial3/DiffPeaks/log_manorm.txt
print("done")
```

```
done
```

The above command will write out several files including the differential peaks for each sample as well as the unchanged peaks.

[11]:
```
!ls Tutorial3/DiffPeaks/output_filters
```

```
CTL_vs_Mutant_M_above_1.0_biased_peaks.bed   CTL_vs_Mutant_unbiased_peaks.bed
CTL_vs_Mutant_M_below_-1.0_biased_peaks.bed
```

[12]:
```
#Let's also check the format of these files
!head Tutorial3/DiffPeaks/output_filters/CTL_vs_Mutant_M_above_1.0_biased_peaks.
 ↪bed
```

```
chr4    52059325        52059732        CTL_unique      2.20155
chr4    52298589        52298799        CTL_unique      1.09775
chr4    52550105        52550494        CTL_unique      1.29536
chr4    52698223        52698464        CTL_unique      1.84416
chr4    52834103        52834470        CTL_unique      1.26119
chr4    52884232        52884622        CTL_unique      1.09835
chr4    52968329        52968671        CTL_unique      1.41519
chr4    52993914        52994157        CTL_unique      1.22576
chr4    53595301        53595477        CTL_unique      1.20393
chr4    53702525        53703113        CTL_unique      1.07373
```

[13]:
```
#We can also count how many are in each.
!wc -l Tutorial3/DiffPeaks/output_filters/*bed
```

```
  124
Tutorial3/DiffPeaks/output_filters/CTL_vs_Mutant_M_above_1.0_biased_peaks.bed
   74
Tutorial3/DiffPeaks/output_filters/CTL_vs_Mutant_M_below_-1.0_biased_peaks.bed
  590 Tutorial3/DiffPeaks/output_filters/CTL_vs_Mutant_unbiased_peaks.bed
  788 total
```

[14]:
```
#Our log file tells us this information as well
!tail Tutorial3/DiffPeaks/log_manorm.txt
```

```
==== Stats ====
Total read pairs of sample 1: 167,920
Total read pairs of sample 2: 219,380
Total peaks of sample 1: 650 (unique: 277 common: 373)
Total peaks of sample 2: 560 (unique: 190 common: 370)
Number of merged common peaks: 369
M-A model: M = -0.04460 * A +0.18904
590 peaks are filtered as unbiased peaks
124 peaks are filtered as sample1-biased peaks
74 peaks are filtered as sample2-biased peaks
```

Annotating Peaks

Let's take the differential peaks and annotate them with nearby genes and perform gene ontology using homer.

First we need to reformat the differential peaks file to the format required by homer.

In an earlier command, we examined the format of manorm's ouput using head and saw that it outputs a five column format. We will change this to a 6 column bed format including a unique name for each peak.

```
[15]: #This command will reformat the peaks file including the line number in naming␣
       ↪the peaks (NR) as well as a place-holder strand in the 6th column (note that␣
       ↪peaks don't necessarily have a strand, but the format requires this column).␣
       ↪The -F \t tells awk that the file is tab delimited.
      !awk '{print $1"\t"$2"\t"$3"\t"$4"_"NR"\t"$5"\t+"}' Tutorial3/DiffPeaks/
       ↪output_filters/CTL_vs_Mutant_M_above_1.0_biased_peaks.bed > Tutorial3/
       ↪GenomeAnnotation/CTL_specific_peaks.bed
      #Let's head this to compare
      !head Tutorial3/GenomeAnnotation/CTL_specific_peaks.bed
```

```
chr4    52059325        52059732        CTL_unique_1    2.20155 +
chr4    52298589        52298799        CTL_unique_2    1.09775 +
chr4    52550105        52550494        CTL_unique_3    1.29536 +
chr4    52698223        52698464        CTL_unique_4    1.84416 +
chr4    52834103        52834470        CTL_unique_5    1.26119 +
chr4    52884232        52884622        CTL_unique_6    1.09835 +
chr4    52968329        52968671        CTL_unique_7    1.41519 +
chr4    52993914        52994157        CTL_unique_8    1.22576 +
chr4    53595301        53595477        CTL_unique_9    1.20393 +
chr4    53702525        53703113        CTL_unique_10   1.07373 +
```

Now let's configure homer to recognize our genome build. We aligned our reads to hg38, so we'll have homer use that.

```
[16]: !perl /opt/conda/share/homer/configureHomer.pl -install hg38 2> Tutorial3/
       ↪DiffPeaks/homer_log1.txt
      print("done")
```

```
done
```

Let's use that reformatted peak file to get nearby genes and perform gene onotology analysis.

```
[19]: !annotatePeaks.pl Tutorial3/GenomeAnnotation/CTL_specific_peaks.bed hg38 -go␣
       ↪Tutorial3/GenomeAnnotation/CTL_GO -annStats Tutorial3/GenomeAnnotation/
       ↪CTL_annStats.txt > Tutorial3/GenomeAnnotation/CTL_specific_Annotated.txt
```

```

        Peak file = Tutorial3/GenomeAnnotation/CTL_specific_peaks.bed
        Genome = hg38
        Organism = human
        Will perform Gene Ontology analysis - output to directory =
Tutorial3/GenomeAnnotation/CTL_GO
        Peak/BED file conversion summary:
                BED/Header formatted lines: 124
                peakfile formatted lines: 0
                Duplicated Peak IDs: 0
        Peak File Statistics:
                Total Peaks: 124
                Redundant Peak IDs: 0
                Peaks lacking information: 0 (need at least 5 columns per peak)
                Peaks with misformatted coordinates: 0 (should be integer)
                Peaks with misformatted strand: 0 (should be either +/- or 0/1)

        Peak file looks good!

        Reading Positions…
        -----------------------
        Finding Closest TSS…
        Annotating:.
                Annotation      Number of peaks Total size (bp) Log2 Ratio
(obs/exp)    LogP enrichment (+values depleted)
                3UTR    0.0     1226327 -0.852  0.802
                miRNA   0.0     3258    -0.003  0.002
                ncRNA   0.0     315963  -0.271  0.206
                TTS     1.0     1306500 0.231   -0.554
                pseudo  0.0     40049   -0.037  0.026
                Exon    0.0     1490268 -0.985  0.975
                Intron  33.0    73166083        -0.532  5.597
                Intergenic      84.0    111121736       0.213   -3.865
                Promoter        5.0     1403974 2.450   -6.038
                5UTR    1.0     114432  3.745   -2.632
        NOTE: If this part takes more than 2 minutes, there is a good chance
                your machine ran out of memory: consider hitting ctrl+C and
rerunning
                the command with "-noann"
        Annotating:.
                Annotation      Number of peaks Total size (bp) Log2 Ratio
(obs/exp)    LogP enrichment (+values depleted)
                3UTR    0.0     1226327 -0.852  0.802
                Retroposon      0.0     200838  -0.178  0.131
                RC?     0.0     2850    -0.003  0.002
                RNA     0.0     6910    -0.006  0.005
                miRNA   0.0     3258    -0.003  0.002
                ncRNA   0.0     315963  -0.271  0.206
                TTS     1.0     1306500 0.232   -0.554
                LINE    18.0    45014382        -0.705  4.777
                srpRNA  0.0     13945   -0.013  0.009
                SINE    6.0     18936280        -1.041  3.470
                RC      0.0     20949   -0.020  0.014
                tRNA    0.0     2852    -0.003  0.002
                DNA?    0.0     27318   -0.025  0.018
                pseudo  0.0     40049   -0.037  0.026
                DNA     1.0     6724040 -2.132  2.750
                Exon    0.0     1490268 -0.985  0.975
                Intron  25.0    38474934        -0.005  0.605
                Intergenic      39.0    48657251        0.298   -2.484
                Promoter        5.0     1403974 2.450   -6.038
                5UTR    1.0     114432  3.745   -2.632
                LTR?    0.0     92183   -0.084  0.060
                scRNA   0.0     6881    -0.006  0.004
                CpG-Island      0.0     373419  -0.315  0.244
                Low_complexity  0.0     365373  -0.309  0.238
                LTR     27.0    20757981        0.997   -7.957
                Simple_repeat   1.0     2265635 -0.563  0.572
                snRNA   0.0     18664   -0.017  0.012
                Unknown 0.0     49238   -0.046  0.032
                SINE?   0.0     130     -0.000  0.000
                Satellite       0.0     2306654 -1.335  1.513
                rRNA    0.0     7760    -0.007  0.005
        Performing Gene Ontology Analysis…
rm: cannot remove '0.353503496709408.bg.tmp': No such file or directory
        Counting Tags in Peaks from each directory…
        Organism: human
        Loading Gene Informaiton…
        Outputing Annotation File…
        Done annotating peaks file
```

Let's look at the output files. First, let's look at the first 2 lines of at our annotation stats.

[20]:

```
#Clean up duplicate entries
!sort -u Tutorial3/GenomeAnnotation/CTL_annStats.txt | grep -v Annotation >␣
 ↪Tutorial3/GenomeAnnotation/CTL_annStats_clean.txt

#Load results into a pandas table
annstats = pd.read_csv("Tutorial3/GenomeAnnotation/CTL_annStats_clean.txt",␣
 ↪sep='\t', header=None,␣
 ↪names=['annotation','peakcount','size','foldenrichment','log10significance'])

#View entries sorted by enrichment
annstats_sorted = annstats.sort_values(by=["foldenrichment"], ascending=False)
display(annstats_sorted)
```

```
        annotation  peakcount       size  foldenrichment  log10significance
1             5UTR        1.0     114432           3.745             -2.632
14        Promoter        5.0    1403974           2.450             -6.038
11             LTR       27.0   20757981           0.997             -7.957
6       Intergenic       39.0   48657251           0.298             -2.484
24             TTS        1.0    1306500           0.232             -0.554
23             TTS        1.0    1306500           0.231             -0.554
7       Intergenic       84.0  111121736           0.213             -3.865
```

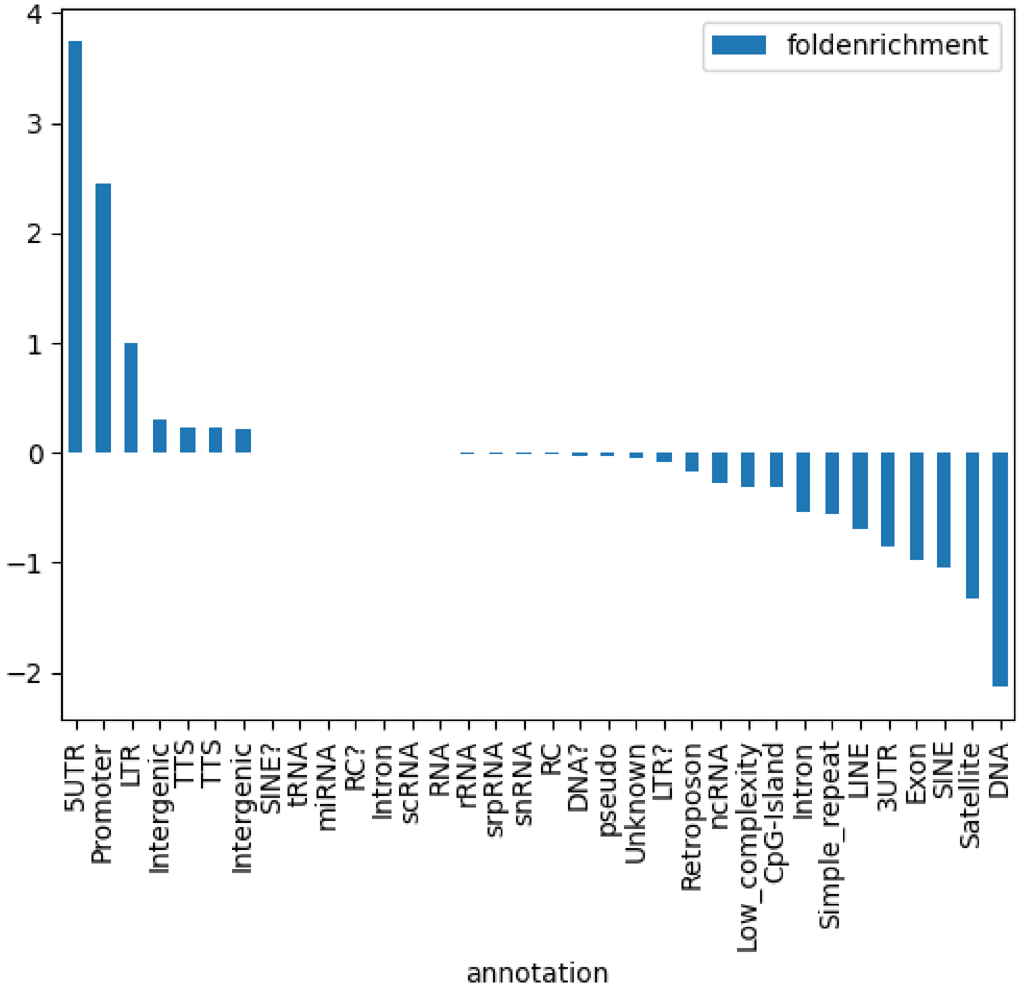
```
20           SINE?        0.0        130          -0.000              0.000
33            tRNA        0.0       2852          -0.003              0.002
26           miRNA        0.0       3258          -0.003              0.002
16             RC?        0.0       2850          -0.003              0.002
8           Intron       25.0   38474934          -0.005              0.605
30           scRNA        0.0       6881          -0.006              0.004
17             RNA        0.0       6910          -0.006              0.005
29            rRNA        0.0       7760          -0.007              0.005
32          srpRNA        0.0      13945          -0.013              0.009
31           snRNA        0.0      18664          -0.017              0.012
15              RC        0.0      20949          -0.020              0.014
4             DNA?        0.0      27318          -0.025              0.018
28          pseudo        0.0      40049          -0.037              0.026
25         Unknown        0.0      49238          -0.046              0.032
12            LTR?        0.0      92183          -0.084              0.060
18      Retroposon        0.0     200838          -0.178              0.131
27           ncRNA        0.0     315963          -0.271              0.206
13  Low_complexity        0.0     365373          -0.309              0.238
2       CpG-Island        0.0     373419          -0.315              0.244
9           Intron       33.0   73166083          -0.532              5.597
22   Simple_repeat        1.0    2265635          -0.563              0.572
10            LINE       18.0   45014382          -0.705              4.777
0             3UTR        0.0    1226327          -0.852              0.802
5             Exon        0.0    1490268          -0.985              0.975
19            SINE        6.0   18936280          -1.041              3.470
21       Satellite        0.0    2306654          -1.335              1.513
3              DNA        1.0    6724040          -2.132              2.750
```

From this we can see highest enrichment in 5’ UTRs and promoters.

Let’s plot the results as a barplot.

```
[21]: annstats_sorted.plot.bar(x="annotation", y="foldenrichment")
```

```
[21]: <Axes: xlabel='annotation'>
```



Homer also outputs the nearest annotation for each peak. Let's look at the first few lines of our annotation file.

[22]:
```
!head -4 Tutorial3/GenomeAnnotation/CTL_specific_Annotated.txt
```

```
PeakID (cmd=annotatePeaks.pl Tutorial3/GenomeAnnotation/CTL_specific_peaks.bed
hg38 -go Tutorial3/GenomeAnnotation/CTL_GO -annStats
Tutorial3/GenomeAnnotation/CTL_annStats.txt)        Chr     Start    End
Strand  Peak Score      Focus Ratio/Region Size Annotation      Detailed
Annotation      Distance to TSS Nearest PromoterID      Entrez ID       Nearest
Unigene Nearest Refseq  Nearest Ensembl Gene Name       Gene Alias      Gene
Description         Gene Type
CTL_unique_56   chr4    64144792        64145494        +       3.01611 NA
Intergenic      HERVK11-int|LTR|ERVK    264307  NM_001010874    253017
Hs.227752       NM_001010874    ENSG00000205678 TECRL
CPVT3|GPSN2L|SRD5A2L2|TERL      trans-2,3-enoyl-CoA reductase like      protein-
coding
```

```
merged_common_90        chr4    54545647        54546360        +       2.38644
NA      Intergenic      Intergenic      61128   NR_134657       339978
NR_134657       ENSG00000250456 LINC02260       -       long intergenic non-
protein coding RNA 2260     ncRNA
CTL_unique_51   chr4    62835674        62836120        +       2.34453 NA
Intergenic      Intergenic      -674132 NR_110595       101927186
Hs.723269       NR_110595               ADGRL3-AS1      LPHN3-AS1       adhesion
G protein-coupled receptor L3 antisense RNA 1  ncRNA
```

Lastly, let's take a look at the gene ontology results

[23]:
```
#list the files in our GO directory
!ls Tutorial3/GenomeAnnotation/CTL_GO/
```

```
biocyc.txt              interactions.txt         prints.txt
biological_process.txt  interpro.txt             prosite.txt
cellular_component.txt  kegg.txt                 reactome.txt
chromosome.txt          lipidmaps.txt            smart.txt
cosmic.txt              molecular_function.txt   smpdb.txt
gene3d.txt              msigdb.txt               wikipathways.txt
geneOntology.html       pathwayInteractionDB.txt
gwas.txt                pfam.txt
```

Let's view the top terms in the biological_process category.

[24]:
```
bp_GO = pd.read_csv("Tutorial3/GenomeAnnotation/CTL_GO/biological_process.txt",␣
 ↪sep='\t')

#keep most significant
bp_GO_top10 = bp_GO.nsmallest(10, "logP")
display(bp_GO_top10)
```

```
       TermID                                              Term  \
0  GO:0052695                           cellular glucuronidation
1  GO:0019585                      glucuronate metabolic process
2  GO:0006063                      uronic acid metabolic process
3  GO:0010817                       regulation of hormone levels
4  GO:0006068                          ethanol catabolic process
5  GO:0034310                  primary alcohol catabolic process
6  GO:0051923                                          sulfation
7  GO:0006067                          ethanol metabolic process
8  GO:0050427  3'-phosphoadenosine 5'-phosphosulfate metaboli…
9  GO:0034035  purine ribonucleoside bisphosphate metabolic p…

     Enrichment       logP  Genes in Term  Target Genes in Term  \
0  8.476896e-07 -13.980751             18                     3
1  1.833506e-06 -13.209280             23                     3
2  1.833506e-06 -13.209280             23                     3
3  1.370570e-05 -11.197699            528                     6
4  7.141780e-05  -9.546963             12                     2
```

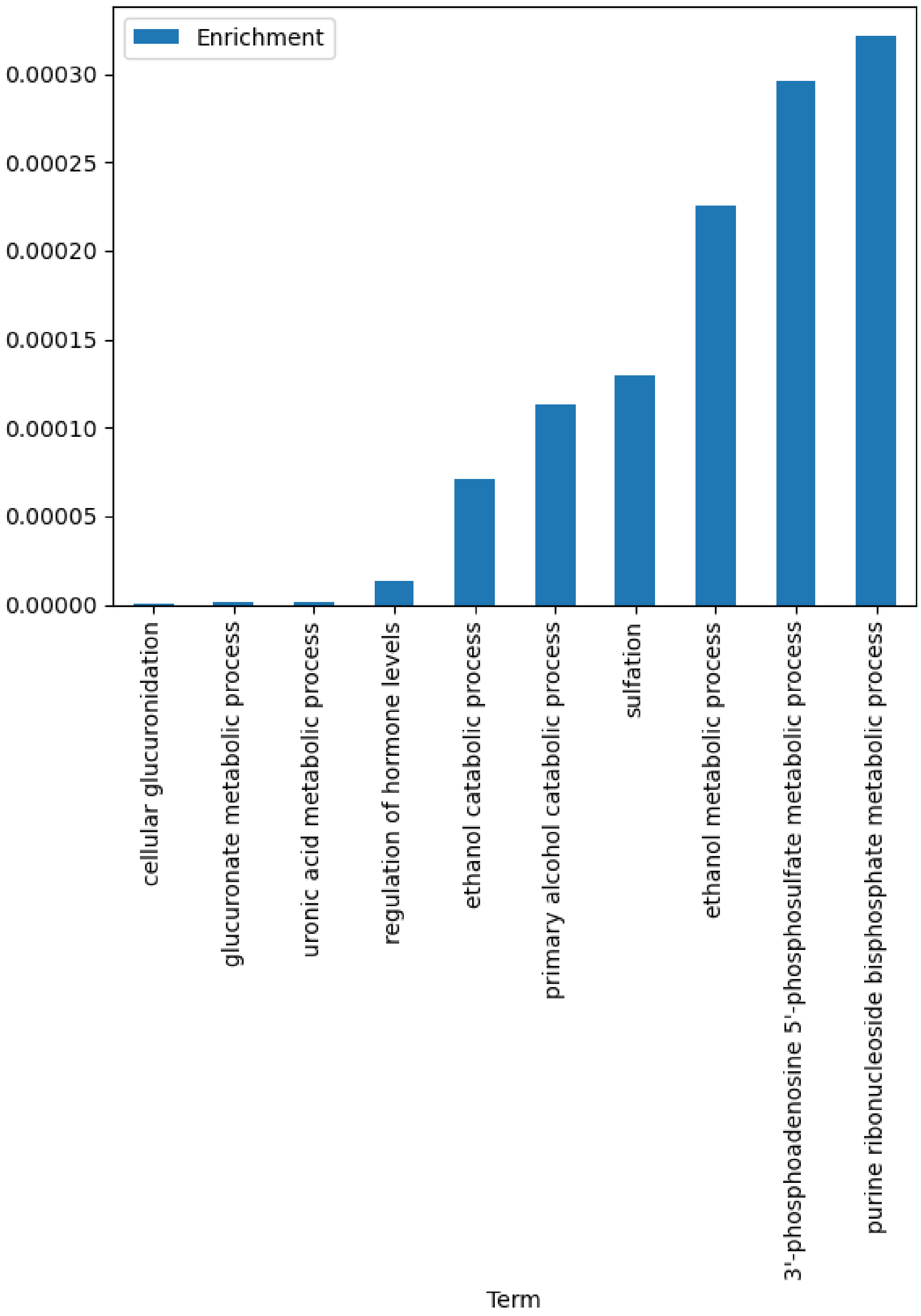
```
5  1.134005e-04  -9.084585           15                2
6  1.295173e-04  -8.951696           16                2
7  2.259286e-04  -8.395291           21                2
8  2.963633e-04  -8.123925           24                2
9  3.219272e-04  -8.041185           25                2

   Fraction of Targets in Term  Total Target Genes  Total Genes  \
0                         0.15                  20        18680
1                         0.15                  20        18680
2                         0.15                  20        18680
3                         0.30                  20        18680
4                         0.10                  20        18680
5                         0.10                  20        18680
6                         0.10                  20        18680
7                         0.10                  20        18680
8                         0.10                  20        18680
9                         0.10                  20        18680

                Entrez Gene IDs                             Gene Symbols
0              79799,7364,10941                     UGT2A3,UGT2B7,UGT2A1
1              10941,79799,7364                     UGT2A1,UGT2A3,UGT2B7
2              79799,7364,10941                     UGT2A3,UGT2B7,UGT2A1
3  5978,7364,2044,6783,9575,27284  REST,UGT2B7,EPHA5,SULT1E1,CLOCK,SULT1B1
4                    27284,6783                          SULT1B1,SULT1E1
5                    27284,6783                          SULT1B1,SULT1E1
6                    6783,27284                          SULT1E1,SULT1B1
7                    27284,6783                          SULT1B1,SULT1E1
8                    27284,6783                          SULT1B1,SULT1E1
9                    27284,6783                          SULT1B1,SULT1E1
```

We can also plot the enrichment scores

Note that our results may look a little odd because we have severely downsampled the data to run quickly and focus on a single region of chr4.

[25]:
```
bp_GO_top10.plot.bar(x="Term", y="Enrichment")
```

[25]:
```
<Axes: xlabel='Term'>
```


Enrichment
0.00030
0.00025
0.00020
0.00015
0.00010
0.00005
0.00000
cellular glucuronidation
glucuronate metabolic process
uronic acid metabolic process
regulation of hormone levels
ethanol catabolic process
primary alcohol catabolic process
sulfation
ethanol metabolic process
3'-phosphoadenosine 5'-phosphosulfate metabolic process
purine ribonucleoside bisphosphate metabolic process
Term

Homer also saves an html file where you can navigate through the various categories.

[26]:
```
#View the html results
IFrame(src='Tutorial3/GenomeAnnotation/CTL_GO/geneOntology.html', width=900, height=600)
```

[26]:
```
<IPython.lib.display.IFrame at 0x7fc16e906ad0>
```

In the above html you can click throught the different ontology categories to view enriched terms and scores for genes near our differential peaks. Note that there are links to motifs, but these lead to "pages not found" because we have yet to do this analysis. We will run motif analysis in the next section using TOBIAS.

Motif Footprinting

1.2.3 ATAC-seq can be used to identify accessibility at transcription factor (TF) binding sites. We'll use tobias.

From: Bentsen et al., Nat. Comm. 2020

Tn5 insertion during ATAC-seq has a sequence bias. In our first step, let's correct for that bias.

[27]:
```
#Index the bam
!samtools index Tutorial3/InputFiles/CTL_dedup.bam
!samtools index Tutorial3/InputFiles/Mutant_dedup.bam
#Tn5 has an insertion sequence bias, which Tobias can correct for. Let's use the master list of peaks provided by manorm, but we need to first remove the header and extra columns.
!cat Tutorial3/DiffPeaks/CTL_vs_Mutant_all_MAvalues.xls | cut -f 1-3 | grep -v start > Tutorial3/MotifFootprinting/MasterPeakList.bed

#Now let's do the signal correction
!TOBIAS ATACorrect --bam Tutorial3/InputFiles/CTL_dedup.bam --genome Tutorial3/InputFiles/chr4.fa --peaks Tutorial3/MotifFootprinting/MasterPeakList.bed --outdir Tutorial3/MotifFootprinting --prefix CTL --cores $numthreadsint --verbosity 1
#Let's also do this for the mutant
!TOBIAS ATACorrect --bam Tutorial3/InputFiles/Mutant_dedup.bam --genome Tutorial3/InputFiles/chr4.fa --peaks Tutorial3/MotifFootprinting/MasterPeakList.bed --outdir Tutorial3/MotifFootprinting --prefix Mutant --cores $numthreadsint --verbosity 1

print("done")
```

```
# TOBIAS 0.16.0 ATACorrect (run started 2023-09-10 04:21:57.812793)
# Working directory: /home/jupyter
# Command line call: TOBIAS ATACorrect --bam Tutorial3/InputFiles/CTL_dedup.bam
--genome Tutorial3/InputFiles/chr4.fa --peaks
Tutorial3/MotifFootprinting/MasterPeakList.bed --outdir
Tutorial3/MotifFootprinting --prefix CTL --cores 7 --verbosity 1
```

```
# ----- Input parameters -----
# bam:  Tutorial3/InputFiles/CTL_dedup.bam
# genome:       Tutorial3/InputFiles/chr4.fa
# peaks:        Tutorial3/MotifFootprinting/MasterPeakList.bed
# regions_in:   None
# regions_out:  None
# blacklist:    None
# extend:       100
# split_strands:        False
# norm_off:     False
# track_off:    []
# drop_chroms:  ['chrM', 'chrMT', 'M', 'MT', 'Mito']
# k_flank:      12
# read_shift:   [4, -5]
# bg_shift:     100
# window:       100
# score_mat:    DWM
# bias_pkl:     None
# prefix:       CTL
# outdir:       /home/jupyter/Tutorial3/MotifFootprinting
# cores:        7
# split:        100
# verbosity:    1


# ----- Output files -----
# /home/jupyter/Tutorial3/MotifFootprinting/CTL_uncorrected.bw
# /home/jupyter/Tutorial3/MotifFootprinting/CTL_bias.bw
# /home/jupyter/Tutorial3/MotifFootprinting/CTL_expected.bw
# /home/jupyter/Tutorial3/MotifFootprinting/CTL_corrected.bw
# /home/jupyter/Tutorial3/MotifFootprinting/CTL_atacorrect.pdf


2023-09-10 04:21:57 (2986006) [WARNING] No additional chromosomes were removed.
Consider using '--drop-chroms' to remove mitochondrial and/or other unwanted
contigs.
```

```
# TOBIAS 0.16.0 ATACorrect (run started 2023-09-10 04:22:37.508418)
# Working directory: /home/jupyter
# Command line call: TOBIAS ATACorrect --bam
Tutorial3/InputFiles/Mutant_dedup.bam --genome Tutorial3/InputFiles/chr4.fa
--peaks Tutorial3/MotifFootprinting/MasterPeakList.bed --outdir
```

```
Tutorial3/MotifFootprinting --prefix Mutant --cores 7 --verbosity 1
```

```
# ----- Input parameters -----
# bam:  Tutorial3/InputFiles/Mutant_dedup.bam
# genome:       Tutorial3/InputFiles/chr4.fa
# peaks:        Tutorial3/MotifFootprinting/MasterPeakList.bed
# regions_in:   None
# regions_out:  None
# blacklist:    None
# extend:       100
# split_strands:        False
# norm_off:     False
# track_off:    []
# drop_chroms:  ['chrM', 'chrMT', 'M', 'MT', 'Mito']
# k_flank:      12
# read_shift:   [4, -5]
# bg_shift:     100
# window:       100
# score_mat:    DWM
# bias_pkl:     None
# prefix:       Mutant
# outdir:       /home/jupyter/Tutorial3/MotifFootprinting
# cores:        7
# split:        100
# verbosity:    1


# ----- Output files -----
# /home/jupyter/Tutorial3/MotifFootprinting/Mutant_uncorrected.bw
# /home/jupyter/Tutorial3/MotifFootprinting/Mutant_bias.bw
# /home/jupyter/Tutorial3/MotifFootprinting/Mutant_expected.bw
# /home/jupyter/Tutorial3/MotifFootprinting/Mutant_corrected.bw
# /home/jupyter/Tutorial3/MotifFootprinting/Mutant_atacorrect.pdf


2023-09-10 04:22:37 (2988574) [WARNING] No additional chromosomes were removed.
Consider using '--drop-chroms' to remove mitochondrial and/or other unwanted
contigs.
```

```
done
```

Now let's use the bias-corrected bigwig files to calculate footprint scores around peaks

[28]:

```
!TOBIAS ScoreBigwig -s Tutorial3/MotifFootprinting/CTL_corrected.bw -r␣
 ↪Tutorial3/MotifFootprinting/MasterPeakList.bed -o Tutorial3/
 ↪MotifFootprinting/CTL_footprintscores.bw --cores $numthreadsint --verbosity 1

#Let's do the same for our mutant sample
!TOBIAS ScoreBigwig -s Tutorial3/MotifFootprinting/Mutant_corrected.bw -r␣
 ↪Tutorial3/MotifFootprinting/MasterPeakList.bed -o Tutorial3/
 ↪MotifFootprinting/Mutant_footprintscores.bw --cores $numthreadsint␣
 ↪--verbosity 1
```

```
# TOBIAS 0.16.0 ScoreBigwig (run started 2023-09-10 04:24:36.386281)
# Working directory: /home/jupyter
# Command line call: TOBIAS ScoreBigwig -s
Tutorial3/MotifFootprinting/CTL_corrected.bw -r
Tutorial3/MotifFootprinting/MasterPeakList.bed -o
Tutorial3/MotifFootprinting/CTL_footprintscores.bw --cores 7 --verbosity 1

# ----- Input parameters -----
# signal:       Tutorial3/MotifFootprinting/CTL_corrected.bw
# output:       Tutorial3/MotifFootprinting/CTL_footprintscores.bw
# regions:      Tutorial3/MotifFootprinting/MasterPeakList.bed
# score:        footprint
# absolute:     False
# extend:       100
# smooth:       1
# min_limit:    None
# max_limit:    None
# fp_min:       20
# fp_max:       50
# flank_min:    10
# flank_max:    30
# window:       100
# cores:        7
# split:        100
# verbosity:    1


# ----- Output files -----
# Tutorial3/MotifFootprinting/CTL_footprintscores.bw



# TOBIAS 0.16.0 ScoreBigwig (run started 2023-09-10 04:24:50.704446)
# Working directory: /home/jupyter
# Command line call: TOBIAS ScoreBigwig -s
Tutorial3/MotifFootprinting/Mutant_corrected.bw -r
Tutorial3/MotifFootprinting/MasterPeakList.bed -o
Tutorial3/MotifFootprinting/Mutant_footprintscores.bw --cores 7 --verbosity 1
```

```
# ----- Input parameters -----
# signal:       Tutorial3/MotifFootprinting/Mutant_corrected.bw
# output:       Tutorial3/MotifFootprinting/Mutant_footprintscores.bw
# regions:      Tutorial3/MotifFootprinting/MasterPeakList.bed
# score:        footprint
# absolute:     False
# extend:       100
# smooth:       1
# min_limit:    None
# max_limit:    None
# fp_min:       20
# fp_max:       50
# flank_min:    10
# flank_max:    30
# window:       100
# cores:        7
# split:        100
# verbosity:    1


# ----- Output files -----
# Tutorial3/MotifFootprinting/Mutant_footprintscores.bw
```

Now that we have our corrected signal and footprint scores, let's do TF binding site prediciton as well as differential footprinting.

Caution: this step searches throug the signal at every signal location corresponding to motifs in your jaspar file. Here we use all the motifs in the jaspar database. This can take several minutes...

[29]:

```
#First, we'll download the current jaspar motifs
!wget https://jaspar.genereg.net/download/data/2022/CORE/JASPAR2022_CORE_vertebrates_non-redundant_pfms_jaspar.txt -P Tutorial3/MotifFootprinting/

#Next we can calculate statistics for each motif represented in our jaspar motif file. If we list both our CTL and Mutant sample, it will calculate the differential footprint score for us as well.
!TOBIAS BINDetect --motifs Tutorial3/MotifFootprinting/JASPAR2022_CORE_vertebrates_non-redundant_pfms_jaspar.txt --signals Tutorial3/MotifFootprinting/CTL_footprintscores.bw Tutorial3/MotifFootprinting/Mutant_footprintscores.bw --genome Tutorial3/InputFiles/chr4.fa --peaks Tutorial3/MotifFootprinting/MasterPeakList.bed --outdir Tutorial3/MotifFootprinting/DiffMotifs --cond_names CTL Mutant --cores $numthreadsint --verbosity 1
```

```
print("done")
```

```
--2023-09-10 04:28:13--  https://jaspar.genereg.net/download/data/2022/CORE/JASP
AR2022_CORE_vertebrates_non-redundant_pfms_jaspar.txt
Resolving jaspar.genereg.net (jaspar.genereg.net)… 193.60.222.202
Connecting to jaspar.genereg.net (jaspar.genereg.net)|193.60.222.202|:443…
connected.
HTTP request sent, awaiting response… 200 OK
Length: 327864 (320K) [text/plain]
Saving to: ‘Tutorial3/MotifFootprinting/JASPAR2022_CORE_vertebrates_non-
redundant_pfms_jaspar.txt’

JASPAR2022_CORE_ver 100%[===================>] 320.18K   594KB/s    in 0.5s

2023-09-10 04:28:14 (594 KB/s) -
‘Tutorial3/MotifFootprinting/JASPAR2022_CORE_vertebrates_non-
redundant_pfms_jaspar.txt’ saved [327864/327864]

/opt/conda/lib/python3.10/site-
packages/logomaker-0.8-py3.10.egg/logomaker/src/validate.py:98: SyntaxWarning:
"is" with a literal. Did you mean "=="?
  if matrix_type is 'information':
/opt/conda/lib/python3.10/site-
packages/logomaker-0.8-py3.10.egg/logomaker/src/validate.py:104: SyntaxWarning:
"is" with a literal. Did you mean "=="?
  elif matrix_type is 'probability':
# TOBIAS 0.16.0 BINDetect (run started 2023-09-10 04:28:24.496648)
# Working directory: /home/jupyter
# Command line call: TOBIAS BINDetect --motifs
Tutorial3/MotifFootprinting/JASPAR2022_CORE_vertebrates_non-
redundant_pfms_jaspar.txt --signals
Tutorial3/MotifFootprinting/CTL_footprintscores.bw
Tutorial3/MotifFootprinting/Mutant_footprintscores.bw --genome
Tutorial3/InputFiles/chr4.fa --peaks
Tutorial3/MotifFootprinting/MasterPeakList.bed --outdir
Tutorial3/MotifFootprinting/DiffMotifs --cond_names CTL Mutant --cores 7
--verbosity 1

# ----- Input parameters -----
# signals:      ['Tutorial3/MotifFootprinting/CTL_footprintscores.bw',
'Tutorial3/MotifFootprinting/Mutant_footprintscores.bw']
# peaks:        Tutorial3/MotifFootprinting/MasterPeakList.bed
# motifs:       ['Tutorial3/MotifFootprinting/JASPAR2022_CORE_vertebrates_non-
redundant_pfms_jaspar.txt']
# genome:       Tutorial3/InputFiles/chr4.fa
# cond_names:   ['CTL', 'Mutant']
# peak_header:  None
# naming:       name_id
# motif_pvalue: 0.0001
# bound_pvalue: 0.001
# pseudo:       None
# time_series:  False
# skip_excel:   False
# output_peaks: None
# norm_off:     False
# prefix:       bindetect
# outdir:       /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs
# cores:        7
# split:        100
# debug:        False
# verbosity:    1


# ----- Output files -----
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/*/beds/*_CTL_bound.bed
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/*/beds/*_CTL_unbound.bed
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/*/beds/*_Mutant_bound.bed
#
/home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/*/beds/*_Mutant_unbound.bed
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/*/beds/*_all.bed
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/*/plots/*_log2fcs.pdf
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/*/*_overview.txt
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/*/*_overview.xlsx
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/bindetect_distances.txt
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/bindetect_results.txt
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/bindetect_results.xlsx
# /home/jupyter/Tutorial3/MotifFootprinting/DiffMotifs/bindetect_figures.pdf
```

```
done
```

[30]:

```
#View the html results
IFrame(src='Tutorial3/MotifFootprinting/DiffMotifs/bindetect_CTL_Mutant.html', width=900, height=600)
```

[30]:

```
<IPython.lib.display.IFrame at 0x7fc16e907790>
```

In the above html file you can hover over each point to see the motif name and the sequence. This type of plot is a volcano plot showing the differntial signal on the x-axis and the significance values on the y-axis.

For example, the original paper focused on TP63, which is one of our differential dots in the html file.

Let's visualize the averge footprint at TP63 motifs.

[31]:
```
#IFrame(src='Tutorial2/MotifFootprinting/MYBL1_MA0776.1/plots/MYBL1_MA0776.
 ↪1_log2fcs.pdf', width=900, height=600)

#note change to Tutorial3
#!TOBIAS PlotAggregate --TFBS Tutorial3/MotifFootprinting/DiffMotifs/
 ↪TP63_MA0525.2/beds/TP63_MA0525.2_all.bed --signals Tutorial3/
 ↪MotifFootprinting/CTL_corrected.bw Tutorial3/MotifFootprinting/
 ↪Mutant_corrected.bw --output Tutorial3/MotifFootprinting/
 ↪TP63_footprint_compare.png --share_y both --verbosity 1 --plot_boundaries␣
 ↪--flank 60 --smooth 2
!TOBIAS PlotAggregate --TFBS Tutorial3/MotifFootprinting/DiffMotifs/TP63_MA0525.
 ↪2/beds/TP63_MA0525.2_all.bed --signals Tutorial3/MotifFootprinting/
 ↪CTL_corrected.bw Tutorial3/MotifFootprinting/Mutant_corrected.bw --output␣
 ↪Tutorial3/MotifFootprinting/TP63_footprint_compare.png --share_y both␣
 ↪--verbosity 1 --plot_boundaries --flank 60 --smooth 2 --signal-on-x
```

```
# TOBIAS 0.16.0 PlotAggregate (run started 2023-09-10 04:38:00.819069)
# Working directory: /home/jupyter
# Command line call: TOBIAS PlotAggregate --TFBS
Tutorial3/MotifFootprinting/DiffMotifs/TP63_MA0525.2/beds/TP63_MA0525.2_all.bed
--signals Tutorial3/MotifFootprinting/CTL_corrected.bw
Tutorial3/MotifFootprinting/Mutant_corrected.bw --output
Tutorial3/MotifFootprinting/TP63_footprint_compare.png --share_y both
--verbosity 1 --plot_boundaries --flank 60 --smooth 2 --signal-on-x

# ----- Input parameters -----
# TFBS: ['Tutorial3/MotifFootprinting/DiffMotifs/TP63_MA0525.2/beds/TP63_MA0525.
2_all.bed']
# signals:      ['Tutorial3/MotifFootprinting/CTL_corrected.bw',
'Tutorial3/MotifFootprinting/Mutant_corrected.bw']
# regions:      []
# whitelist:    []
# blacklist:    []
# output:       Tutorial3/MotifFootprinting/TP63_footprint_compare.png
# output_txt:   None
# title:        Aggregated signals
# flank:        60
# TFBS_labels:  None
# signal_labels:        None
# region_labels:        None
# share_y:      both
# normalize:    False
# negate:       False
# smooth:       2
# log_transform:        False
# plot_boundaries:      True
# signal_on_x:  True
# remove_outliers:      1
# verbosity:    1


# ----- Output files -----
# Tutorial3/MotifFootprinting/TP63_footprint_compare.png
```

[32]:
```
IFrame(src='Tutorial3/MotifFootprinting/TP63_footprint_compare.png', width=600,␣
 ↪height=400)
```

[32]:
```
<IPython.lib.display.IFrame at 0x7fc16e906f20>
```

We can also get all the motifs that have differential footprints:

[33]:
```
#!load the results as a pandas table Tutorial2/MotifFootprinting/
 ↪bindetect_results.txt
dframe = pd.read_csv("Tutorial3/MotifFootprinting/DiffMotifs/bindetect_results.
 ↪txt", sep='\t')
display(dframe)
DiffMotifs = dframe[dframe['CTL_Mutant_pvalue'] < .05]
#Write out to a tab separated file
DiffMotifs.to_csv('Tutorial3/MotifFootprinting/DiffMotifs_p05.txt')
```

```
           output_prefix          name  motif_id         cluster  total_tfbs  \
0          Arnt_MA0004.1          Arnt  MA0004.1           C_MYC          38
1       AhrArnt_MA0006.1     Ahr::Arnt  MA0006.1     C_Ahr::Arnt          48
2    Ddit3Cebpa_MA0019.1  Ddit3::Cebpa  MA0019.1  C_Ddit3::Cebpa          62
3         Mecom_MA0029.1         Mecom  MA0029.1         C_Mecom          74
4         FOXF2_MA0030.1         FOXF2  MA0030.1         C_FOXD1          70
..                   …             …        …               …         …
836      ZNF281_MA1630.2        ZNF281  MA1630.2        C_ZNF281         442
837       BACH1_MA1633.2         BACH1  MA1633.2          C_JUNB         292
838       Prdm4_MA1647.2         Prdm4  MA1647.2         C_Prdm4          90
839       THAP1_MA0597.2         THAP1  MA0597.2         C_THAP1         236
840       NR5A1_MA1540.2         NR5A1  MA1540.2         C_NR5A1          88

     CTL_mean_score  CTL_bound  Mutant_mean_score  Mutant_bound  \
0          85.97575         16          108.49061             17
1         122.76075         24          115.50042             22
2          80.02321         24           80.35243             23
3          61.72563         20           58.21735             21
4          54.90034         16           57.53936             12
..              …        …                …           …
836        87.47115        177           93.39986            192
837       107.33995        180           93.78144            132
838        65.86800         28           60.37410             24
839        90.57476        101           82.80139             92
840        60.86295         20           63.31876             24

     CTL_Mutant_change  CTL_Mutant_pvalue  CTL_Mutant_highlighted
0             -0.37973       7.880020e-46                    True
1              0.16950       1.263900e-19                   False
2              0.01402       3.506450e-01                   False
3              0.04204       6.714740e-03                   False
4              0.02603       1.081680e-01                   False
..                 …                …                     …
836           -0.09869       3.777940e-38                   False
837            0.44457       1.482660e-86                    True
838            0.21494       1.058610e-39                   False
839            0.16366       5.913700e-48                   False
840            0.00371       2.191130e-01                   False

[841 rows x 12 columns]
```

Great job!

Thank you for completing these tutorials. Feel free to download these notebooks, customize, and use them to process your own data.

[ ]: