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DSIB01 Autumn 2021

05 Motif Detection

Overview

- Peak calling - brief overview
- Motif representation in biology
  - PPM
  - PWM
  - sequence logos
- Tools
  - Bedops
  - Bedtools
  - The MEME Suite
    - MEME-ChIP
    - Tomtom
- Demo on real dataset
- Homework - Individual work

Clip-seq analysis - peak calling

- a statistical procedure, which uses coverage properties of CLIP and Input samples to find regions which are enriched due to protein binding
- requires mapped reads, and outputs a set of regions, which represent the putative binding locations. Each region is usually associated with a significance score which is an indicator of enrichment
- many different tools for peak calling available:
  - **iCount**
  - **Paraclu**
  - **PureCLIP**
  - **Piranha**

Sequence motifs

- a **nucleotide or amino-acid sequence pattern** that is widespread and usually assumed to be **related to biological function** of the macromolecule

- **short, recurring patterns** in DNA/RNA that are presumed to have a biological function. Often they i**ndicate sequence-specific binding sites** for proteins such as nucleases, transcription factors, RNA-binding proteins. Others are **involved in important processes** at the RNA level, including ribosome binding, mRNA processing (splicing, editing, polyadenylation) and transcription termination.

Sequence motif representation - PPMs

- a **position probability matrix**
- in general:
  - there’s one row for each symbol of the alphabet and one column for each position in the pattern
- in **PPM** each number is a **probability of nucleotide** occurrence **in given position** (sum of each column is 1)

$$M = \begin{array}{c} A \\ C \\ G \\ T \end{array} \begin{bmatrix} 0.3 & 0.6 & 0.1 & 0.0 & 0.0 & 0.6 & 0.7 & 0.2 & 0.1 \\ 0.2 & 0.2 & 0.1 & 0.0 & 0.0 & 0.2 & 0.1 & 0.1 & 0.2 \\ 0.1 & 0.1 & 0.7 & 1.0 & 0.0 & 0.1 & 0.1 & 0.5 & 0.1 \\ 0.4 & 0.1 & 0.1 & 0.0 & 1.0 & 0.1 & 0.1 & 0.2 & 0.6 \end{bmatrix}.$$

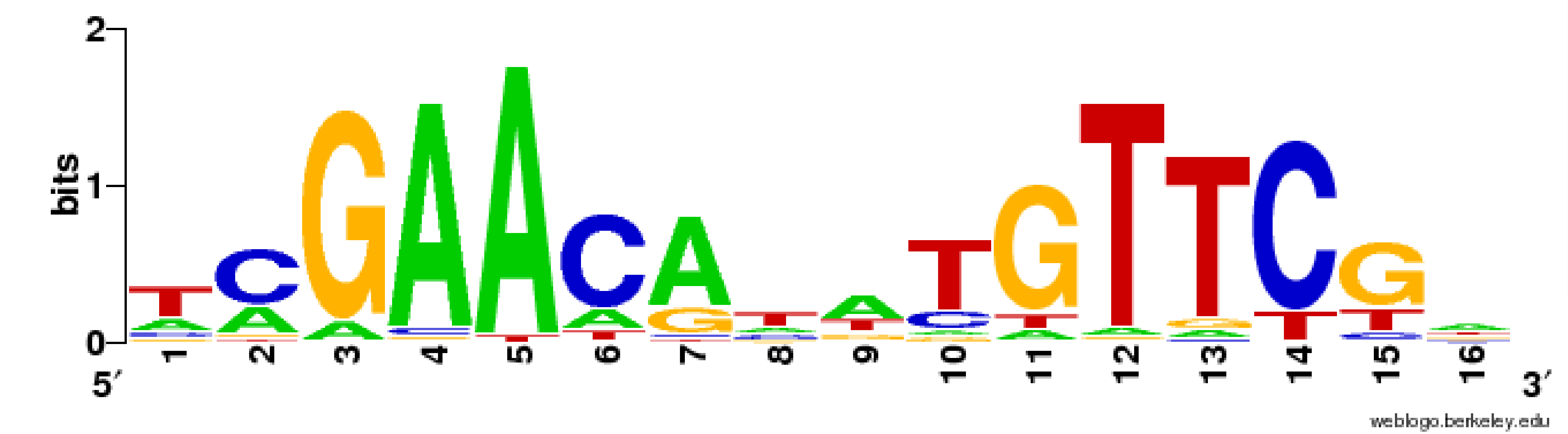
Sequence motif representation - PWMs

- a **position weight matrix**
  - also known as a position-specific weight matrix (**PSWM**) or position-specific scoring matrix (**PSSM**)
  - **the most commonly used**
- the elements in PWMs are calculated as **log likelihoods**
- PWMs are often derived from a set of aligned sequences that are thought to be functionally related and have become an important part of many software tools for computational motif discovery.

$$M = \begin{array}{c} A \\ C \\ G \\ T \end{array} \begin{bmatrix} 0.26 & 1.26 & -1.32 & -\infty & -\infty & 1.26 & 1.49 & -0.32 & -1.32 \\ -0.32 & -0.32 & -1.32 & -\infty & -\infty & -0.32 & -1.32 & -1.32 & -0.32 \\ -1.32 & -1.32 & 1.49 & 2.0 & -\infty & -1.32 & -1.32 & 1.0 & -1.32 \\ 0.68 & -1.32 & -1.32 & -\infty & 2.0 & -1.32 & -1.32 & -0.32 & 1.26 \end{bmatrix}.$$

Sequence motif representation - Sequence logos

- Graphical representation of PWMs
  - **the bigger letter the higher chance for the nucleotide to appear in the position**



Tools - BEDOPS + bedtools

- **BEDOPS:**
  - open-source command-line toolkit that performs efficient and scalable Boolean and other set operations, statistical calculations, archiving, conversion and other management of genomic data of arbitrary scale
  - https://bedops.readthedocs.io/en/latest/
  - functions for today: **sort-bed**, **bedextract**
- **bedtools:**
  - a swiss-army knife of tools for a wide-range of genomics analysis tasks
  - allows one to intersect, merge, count, complement, and shuffle genomic intervals from multiple files and in many different formats (.bed, .bam, .gff, …)
  - https://bedtools.readthedocs.io/en/latest/
  - function for today: **getfasta**

Tools - The MEME Suite

- **The MEME Suite** is a powerful, integrated set of web-based tools for studying sequence motifs in proteins, DNA and RNA.

- **MEME-ChIP**
  - web service designed to analyze ChIP-seq ‘peak regions’ - short genomic regions surrounding declared ChIP-seq ‘peaks’
  - works also with **CLIP-seq** ‘peak regions’
  - Given a set of genomic regions, it performs:
    - ab initio motif discovery
    - motif enrichment analysis
    - motif visualization
    - binding affinity analysis
    - motif identification
  - https://meme-suite.org/meme/tools/meme-chip

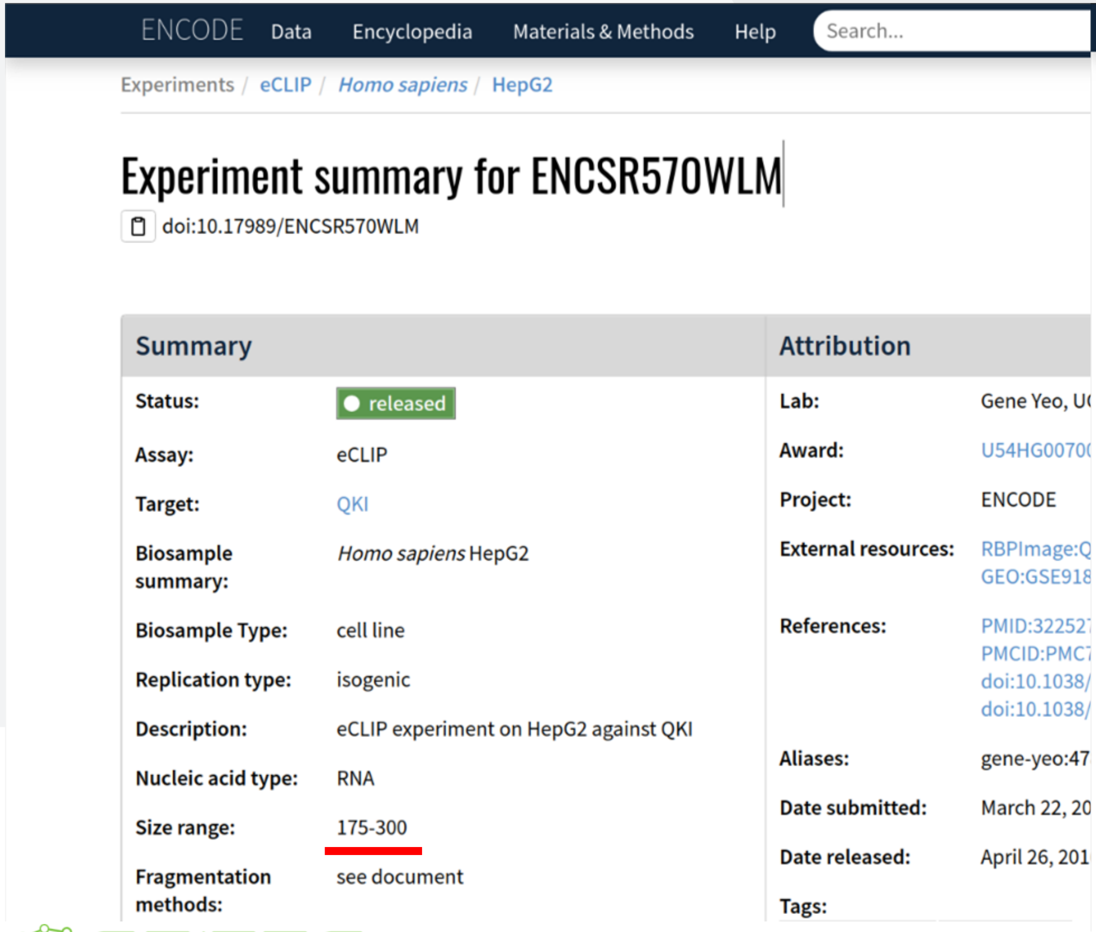
Tools - The MEME Suite

- The MEME Suite is a powerful, integrated set of web-based tools for studying sequence motifs in proteins, DNA and RNA.

- **Tomtom**
  - web service that allows the user to **compare motifs** discovered by the suite, by other tools, or taken from the literature to all of the motifs in a selected database of motifs
  - aligns each input motif with each motif in the selected database and reports the most similar pairs, along with estimates of the statistical significance of each match
  - https://meme-suite.org/meme/tools/tomtom

Real dataset

1. Download the dataset: bed file with peaks, choose isogenic replicate 1,2
   https://www.encodeproject.org/experiments/ENCSR570WLM/
2. Download the chromosome 1 fasta reference
3. Unzip the files



Experiments / eCLIP / *Homo sapiens* / HepG2

Experiment summary for ENCSR570WLM

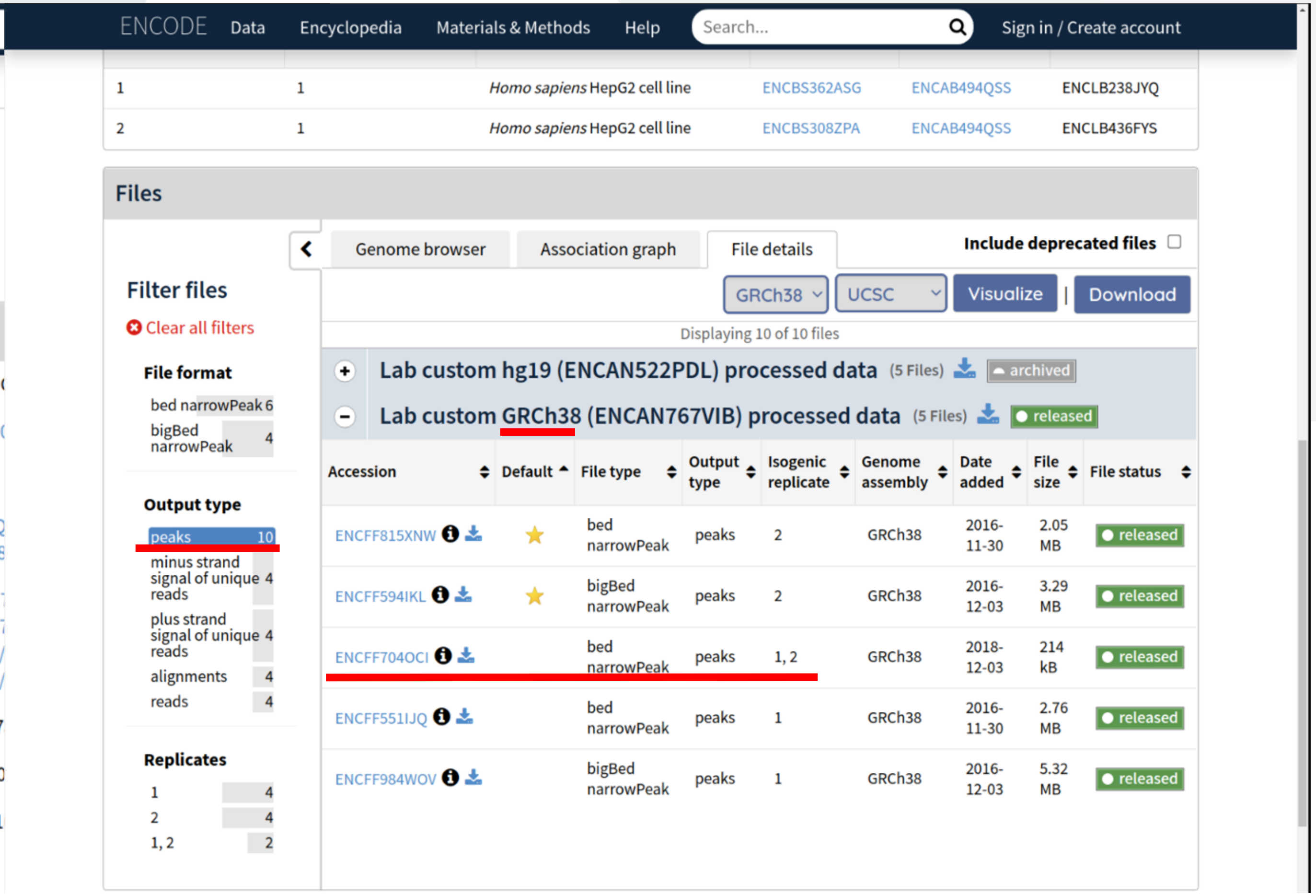
doi:10.17989/ENCSR570WLM

Summary

Status:	● released
Assay:	eCLIP
Target:	QKI
Biosample summary:	*Homo sapiens* HepG2
Biosample Type:	cell line
Replication type:	isogenic
Description:	eCLIP experiment on HepG2 against QKI
Nucleic acid type:	RNA
Size range:	175-300
Fragmentation methods:	see document

Attribution

Lab:	Gene Yeo, U(
Award:	U54HG0070(
Project:	ENCODE
External resources:	RBPImage:Q GEO:GSE918
References:	PMID:32252 PMCID:PMC7 doi:10.1038/ doi:10.1038/
Aliases:	gene-yeo:47
Date submitted:	March 22, 20
Date released:	April 26, 201
Tags:	



1	1	*Homo sapiens* HepG2 cell line	ENCBS362ASG	ENCAB494QSS	ENCLB238JYQ
2	1	*Homo sapiens* HepG2 cell line	ENCBS308ZPA	ENCAB494QSS	ENCLB436FYS

Files

Filter files

Clear all filters

File format

bed narrowPeak	6
bigBed narrowPeak	4

Output type

peaks	10
minus strand signal of unique reads	4
plus strand signal of unique reads	4
alignments	4
reads	4

Replicates

1	4
2	4
1, 2	2

Displaying 10 of 10 files

+ Lab custom hg19 (ENCAN522PDL) processed data (5 Files) archived

− Lab custom GRCh38 (ENCAN767VIB) processed data (5 Files) ● released

Accession	Default	File type	Output type	Isogenic replicate	Genome assembly	Date added	File size	File status
ENCFF815XNW	★	bed narrowPeak	peaks	2	GRCh38	2016-11-30	2.05 MB	● released
ENCFF594IKL	★	bigBed narrowPeak	peaks	2	GRCh38	2016-12-03	3.29 MB	● released
ENCFF704OCI		bed narrowPeak	peaks	1, 2	GRCh38	2018-12-03	214 kB	● released
ENCFF551IJQ		bed narrowPeak	peaks	1	GRCh38	2016-11-30	2.76 MB	● released
ENCFF984WOV		bigBed narrowPeak	peaks	1	GRCh38	2016-12-03	5.32 MB	● released

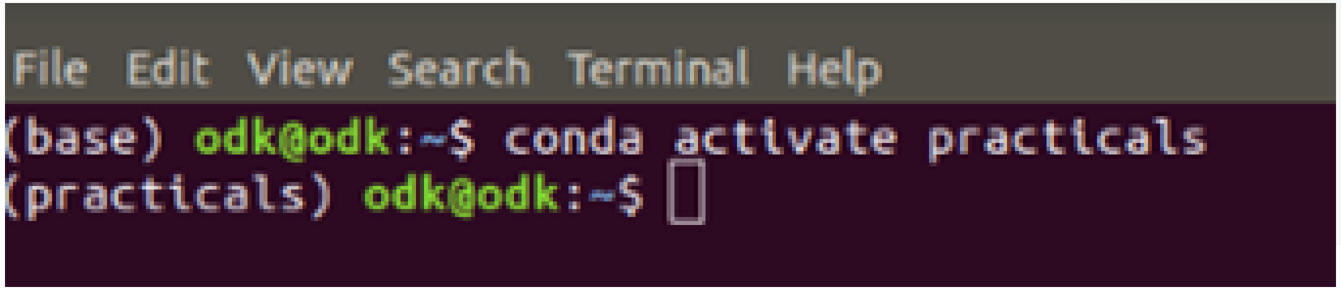
Real dataset

4. Create and activate conda environment for today’s practicals
   - Open the **terminal**

```
conda create --name practicals
conda activate practicals
```

5. Installation of necessary packages:
   - if it turns out you’re missing a channel for installing some of the tool, you can add them by following cmd:

```
conda config --add channels NAME
conda install bedops
conda install –c bioconda bedtools
```

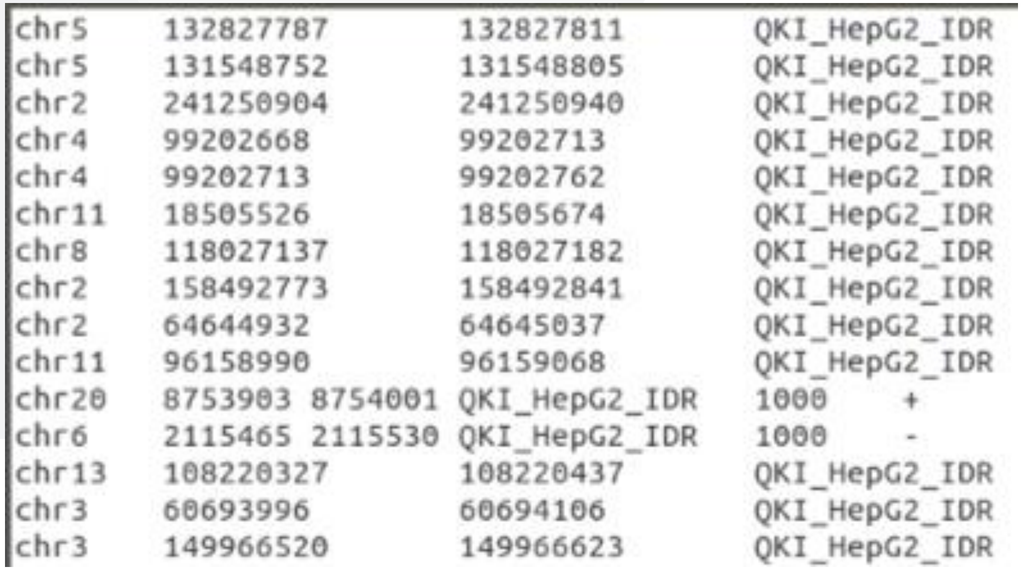


```
(base) odk@odk:~$ conda activate practicals
(practicals) odk@odk:~$ 
```

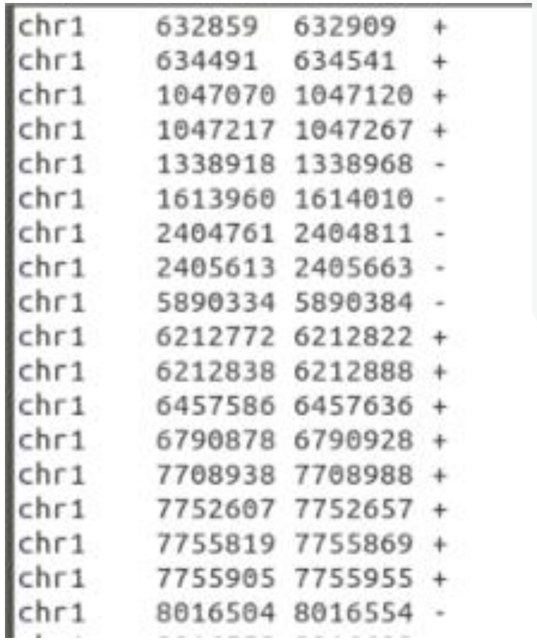
Real dataset

6. Sort intervals in downloaded file and then extract chromosome 1 positions
   - `sort-bed PATH/TO/peaks.bed > PATH/TO/OUTPUT/sorted_peaks.bed`
7. Unify intervals length to 100 nt

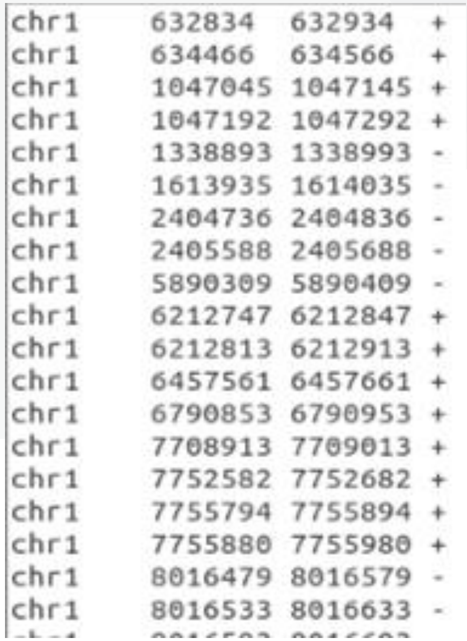
   -
     ```
     awk -F '\t' '{X=50; mid=(int($2)+int($3))/2;printf("%s\t%d\t%d\t%s\n",$1,(mid-X<0?0:mid-X),mid+X, $4);}' PATH/TO/chr1_peaks.bed > PATH/TO/OUTPUT/chr1_peaks_extended.bed
     ```



```
chr5    132827787       132827811       QKI_HepG2_IDR
chr5    131548752       131548805       QKI_HepG2_IDR
chr2    241250904       241250940       QKI_HepG2_IDR
chr4    99202668        99202713        QKI_HepG2_IDR
chr4    99202713        99202762        QKI_HepG2_IDR
chr11   18505526        18505674        QKI_HepG2_IDR
chr8    118027137       118027182       QKI_HepG2_IDR
chr2    158492773       158492841       QKI_HepG2_IDR
chr2    64644932        64645037        QKI_HepG2_IDR
chr11   96158990        96159068        QKI_HepG2_IDR
chr20   8753903 8754001 QKI_HepG2_IDR   1000    +
chr6    2115465 2115530 QKI_HepG2_IDR   1000    -
chr13   108220327       108220437       QKI_HepG2_IDR
chr3    60693996        60694106        QKI_HepG2_IDR
chr3    149966520       149966623       QKI_HepG2_IDR
```



```
chr1    632859  632909  +
chr1    634491  634541  +
chr1    1047070 1047120 +
chr1    1047217 1047267 +
chr1    1338918 1338968 -
chr1    1613960 1614010 -
chr1    2404761 2404811 -
chr1    2405613 2405663 -
chr1    5890334 5890384 -
chr1    6212772 6212822 +
chr1    6212838 6212888 +
chr1    6457586 6457636 +
chr1    6790878 6790928 +
chr1    7708938 7708988 +
chr1    7752607 7752657 +
chr1    7755819 7755869 +
chr1    7755905 7755955 +
chr1    8016504 8016554 -
```

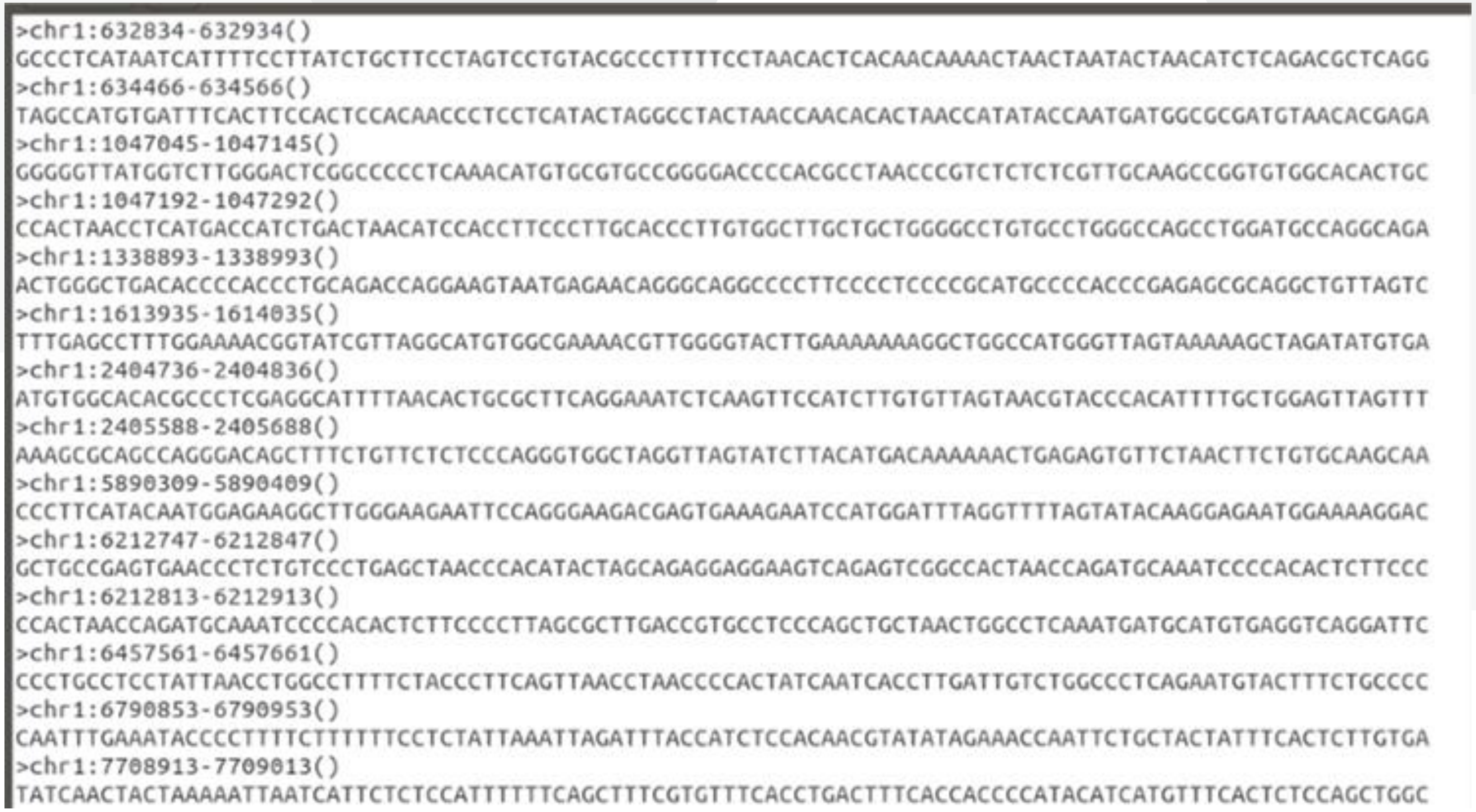


```
chr1    632834  632934  +
chr1    634466  634566  +
chr1    1047045 1047145 +
chr1    1047192 1047292 +
chr1    1338893 1338993 -
chr1    1613935 1614035 -
chr1    2404736 2404836 -
chr1    2405588 2405688 -
chr1    5890309 5890409 -
chr1    6212747 6212847 +
chr1    6212813 6212913 +
chr1    6457561 6457661 +
chr1    6790853 6790953 +
chr1    7708913 7709013 +
chr1    7752582 7752682 +
chr1    7755794 7755894 +
chr1    7755880 7755980 +
chr1    8016479 8016579 -
chr1    8016533 8016633 -
```

Real dataset

8. Extract sequences from a reference FASTA file for each of the intervals

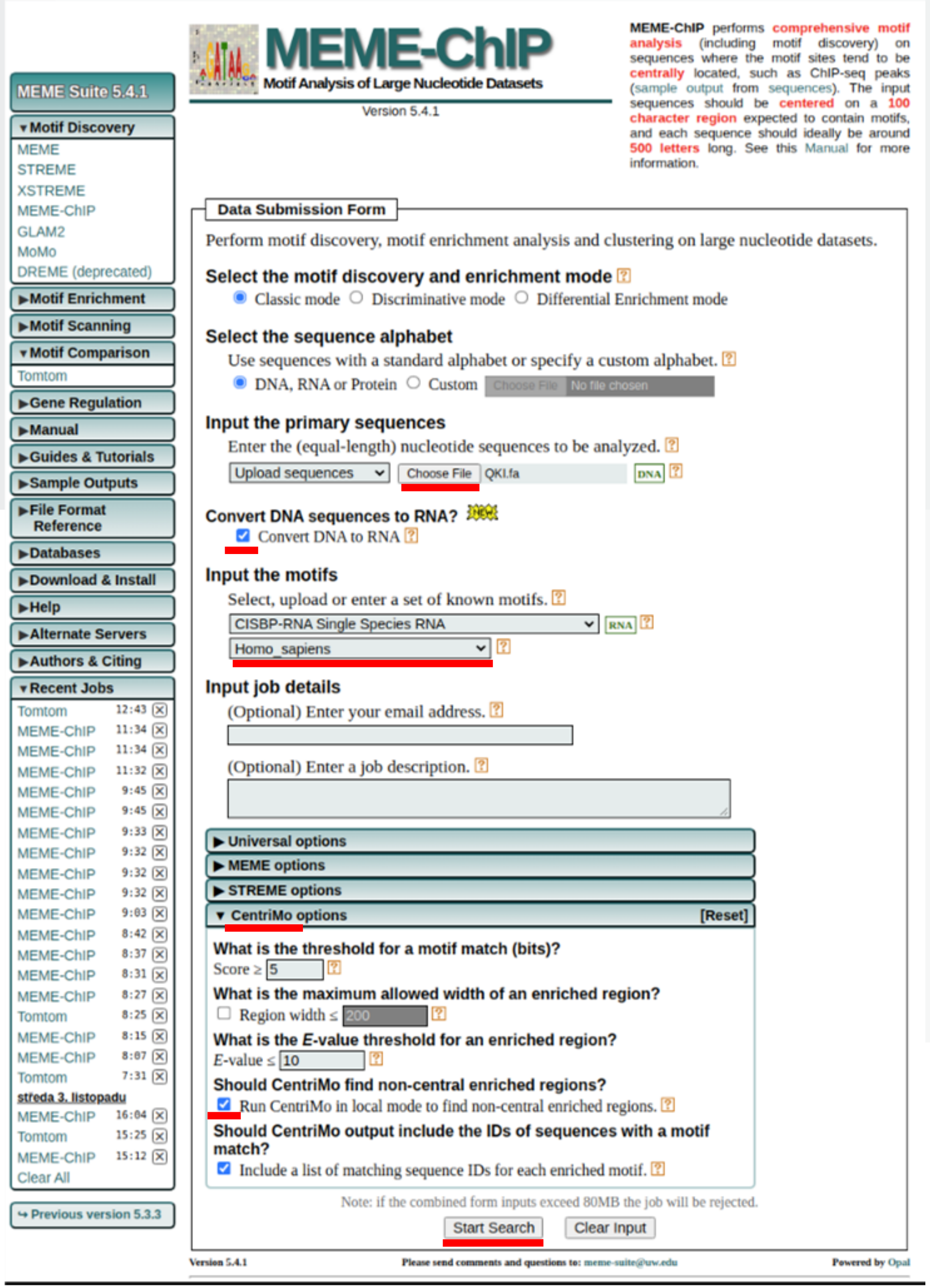
```
bedtools getfasta -s -fi PATH/TO/chr1.fasta -bed PATH/TO/chr1_peaks_extended.bed -fo
PATH/TO/QKI_chr1.fa
```



```
>chr1:632834-632934()
GCCCTCATAATCATTTTCCTTATCTGCTTCCTAGTCCTGTACGCCCTTTTCCTAACACTCACAACAAAACTAACTAATACTAACATCTCAGACGCTCAGG
>chr1:634466-634566()
TAGCCATGTGATTTCACTTCCACTCCACAACCCTCCTCATACTAGGCCTACTAACCAACACACTAACCATATACCAATGATGGCGCGATGTAACACGAGA
>chr1:1047045-1047145()
GGGGGTTATGGTCTTGGGACTCGGCCCCCTCAAACATGTGCGTGCCGGGGACCCCACGCCTAACCCGTCTCTCTCGTTGCAAGCCGGTGTGGCACACTGC
>chr1:1047192-1047292()
CCACTAACCTCATGACCATCTGACTAACATCCACCTTCCCTTGCACCCTTGTGGCTTGCTGCTGGGGCCTGTGCCTGGGCCAGCCTGGATGCCAGGCAGA
>chr1:1338893-1338993()
ACTGGGCTGACACCCCACCCTGCAGACCAGGAAGTAATGAGAACAGGGCAGGCCCCTTCCCCTCCCCGCATGCCCCACCCGAGAGCGCAGGCTGTTAGTC
>chr1:1613935-1614035()
TTTGAGCCTTTGGAAAACGGTATCGTTAGGCATGTGGCGAAAACGTTGGGGTACTTGAAAAAAAGGCTGGCCATGGGTTAGTAAAAAGCTAGATATGTGA
>chr1:2404736-2404836()
ATGTGGCACACGCCCTCGAGGCATTTTAACACTGCGCTTCAGGAAATCTCAAGTTCCATCTTGTGTTAGTAACGTACCCACATTTTGCTGGAGTTAGTTT
>chr1:2405588-2405688()
AAAGCGCAGCCAGGGACAGCTTTCTGTTCTCTCCCAGGGTGGCTAGGTTAGTATCTTACATGACAAAAAACTGAGAGTGTTCTAACTTCTGTGCAAGCAA
>chr1:5890309-5890409()
CCCTTCATACAATGGAGAAGGCTTGGGAAGAATTCCAGGGAAGACGAGTGAAAGAATCCATGGATTTAGGTTTTAGTATACAAGGAGAATGGAAAAGGAC
>chr1:6212747-6212847()
GCTGCCGAGTGAACCCTCTGTCCCTGAGCTAACCCACATACTAGCAGAGGAGGAAGTCAGAGTCGGCCACTAACCAGATGCAAATCCCCACACTCTTCCC
>chr1:6212813-6212913()
CCACTAACCAGATGCAAATCCCCACACTCTTCCCCTTAGCGCTTGACCGTGCCTCCCAGCTGCTAACTGGCCTCAAATGATGCATGTGAGGTCAGGATTC
>chr1:6457561-6457661()
CCCTGCCTCCTATTAACCTGGCCTTTTCTACCCTTCAGTTAACCTAACCCCACTATCAATCACCTTGATTGTCTGGCCCTCAGAATGTACTTTCTGCCCC
>chr1:6790853-6790953()
CAATTTGAAATACCCCTTTTCTTTTTTCCTCTATTAAATTAGATTTACCATCTCCACAACGTATATAGAAACCAATTCTGCTACTATTTCACTCTTGTGA
>chr1:7708913-7709013()
TATCAACTACTAAAAATTAATCATTCTCTCCATTTTTTCAGCTTTCGTGTTTCACCTGACTTTCACCACCCCATACATCATGTTTCACTCTCCAGCTGGC
```

Real dataset

9. Open the [MEME Suite](https://meme-suite.org) web
10. Open the **MEME-ChIP** tool
11. Pick appropriate setup
12. **Run** the analysis



MEME-ChIP

Motif Analysis of Large Nucleotide Datasets

Version 5.4.1

MEME-ChIP performs **comprehensive motif analysis** (including motif discovery) on sequences where the motif sites tend to be **centrally** located, such as ChIP-seq peaks (sample output from sequences). The input sequences should be **centered** on a **100 character region** expected to contain motifs, and each sequence should ideally be around **500 letters** long. See this Manual for more information.

Data Submission Form

Perform motif discovery, motif enrichment analysis and clustering on large nucleotide datasets.

Select the motif discovery and enrichment mode ?

● Classic mode ○ Discriminative mode ○ Differential Enrichment mode

Select the sequence alphabet

Use sequences with a standard alphabet or specify a custom alphabet. ?

● DNA, RNA or Protein ○ Custom Choose File No file chosen

Input the primary sequences

Enter the (equal-length) nucleotide sequences to be analyzed. ?

Upload sequences ⌄ Choose File QKI.fa DNA ?

Convert DNA sequences to RNA? NEW

☑ Convert DNA to RNA ?

Input the motifs

Select, upload or enter a set of known motifs. ?

CISBP-RNA Single Species RNA ⌄ RNA ?

Homo_sapiens ⌄ ?

Input job details

(Optional) Enter your email address. ?

(Optional) Enter a job description. ?

▶ Universal options

▶ MEME options

▶ STREME options

▼ CentriMo options [Reset]

What is the threshold for a motif match (bits)?

Score ≥ 5 ?

What is the maximum allowed width of an enriched region?

☐ Region width ≤ 200 ?

What is the *E*-value threshold for an enriched region?

E-value ≤ 10 ?

Should CentriMo find non-central enriched regions?

☑ Run CentriMo in local mode to find non-central enriched regions. ?

Should CentriMo output include the IDs of sequences with a motif match?

☑ Include a list of matching sequence IDs for each enriched motif. ?

Note: if the combined form inputs exceed 80MB the job will be rejected.

Start Search Clear Input

Version 5.4.1 Please send comments and questions to: meme-suite@uw.edu Powered by Opal

Homework

- **Re-do the motif analysis on the artificial dataset**
- 4 different datasets (1 dataset per student) + 1 bonus dataset
  - will be sent by email
- **Task:**
  - download the data
  - extend the intervals to 100 nt
  - extract sequences for the intervals
  - use MEME-ChIP to analyse motifs in dataset
  - try to identify domain/protein/protein family

    (look also at the CISBP database and pfam database - by clicking through the results)
- **Bonus task 1:**
  - Download the Motifs in MEME Text Format, upload the file to Tomtom tool, choose the CISBP-RNA Single Species RNA (Homo Sapiens) motif database and look at the results of the motif comparison tool
- **Bonus task 2:**
  - Repeat the analysis on the bonus (voluntary) dataset
- **We'll discuss the results on the practicals 3. 12. 2021**