# Replication of ChiP-Seq Analysis

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# Gene induction and repression during terminal erythropoiesis are mediated by distinct epigenetic changes

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It is unclear how epigenetic changes regulate the induction of erythroid-specific genes during terminal erythropoiesis. In this project the researchers used global mRNA sequencing (mRNA-seq) and chromatin immunoprecipitation coupled to high-throughput sequencing (CHIP-seq) to investigate the changes that occur in mRNA levels, RNA polymerase II (Pol II) occupancy, and multiple posttranslational histone modifications when erythroid progenitors differentiate into late erythroblasts. Among genes induced during this developmental transition, there was an increase in the occupancy of Pol II, the activation marks H3K4me2, H3K4me3, H3K9Ac, and H4K16Ac, and the elongation methylation mark H3K79me2. In contrast, genes that were repressed during differentiation showed relative decreases in H3K79me2 levels yet had levels of Pol II binding and active histone marks similar to those in erythroid progenitors.

They also found that relative changes in histone modification levels, in particular, H3K79me2 and H4K16ac, were most predictive of gene expression patterns. The results suggest that in terminal erythropoiesis both promoter and elongation-associated marks contribute to the induction of erythroid genes, whereas gene repression is marked by changes in histone modifications mediating Pol II elongation. The data map the epigenetic landscape of terminal erythropoiesis and suggest that control of transcription elongation regulates gene expression during terminal erythroid differentiation.

**Contents**

**Data**

| Sample Name | GEO Accession | Chip Anti-Body | Project Report Shortname |
|---|---|---|---|
| SRR1157326 | GSM688808 | H3K4me2 | Sample0 |
| SRR1157329 | GSM688811 | H3K27me3 | Sample1 |
| SRR1157333 | GSM688815 | RNA Pol II | Sample2 |
| SRR1157341 | GSM688824 | RNA Pol II | Sample3 |

No control or input/baseline was among the samples selected.

In general, the mouse genome (mm9 version) was used for reference during the stages of Bowtie, MACS, IGV and MEME.

**Tools**

Python 2.7.12

Samtools 1.11 (Utility)

Bedtools 2.27.1 (Utility)

FastQC 0.11.9 (Quality Control)

Minion (Adapter Prediction)

Cutadapt 1.9.1 (Adapter Trimming)

Bowtie 1.0.0 (Alignment)

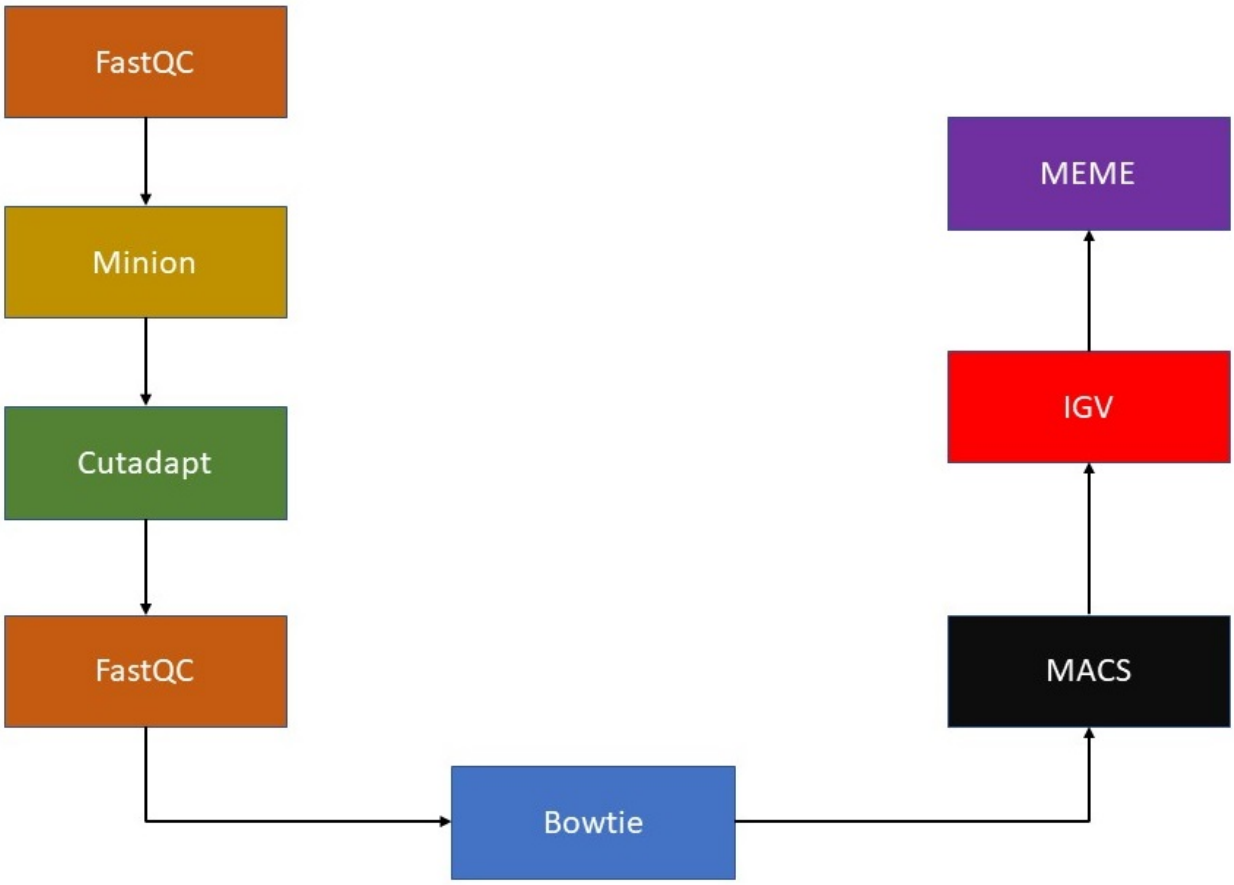
MACS 1.4.1 (Peak Calling)

MEME 5.0.2 (Motif analysis)

**Protocol**

The following steps took place as part of the ChiP-seq analysis:

1. Quality Control (FastQC)
2. Adapter Prediction (Minion)
3. Adapter Trimming (Cutadapt)
4. Quality Control (FastQC)
5. Alignment (Bowtie)
6. Peak Calling (MACS)
7. Visualization (IGV)
8. Motif Analysis (MEME)



You can view the log file of all commands by clicking the link:

Commands Log

These commands were executed for each sample.

**FastQC**

At first, there needed to be some quality control checks on the raw sequence data of the four samples, which would give us a quick impression of whether the data has any problems of which I should be aware before doing any further analysis.

- For Sample1 & Sample3, I observed a failure implying that something is happening in the proportion of each base in each position. The lines should be parallel in normal conditions.
- Furthermore, there was a failure in “Adapter Content”, suggesting that there are adapters in the samples.
- For Sample3, also, the Per base-Per sequence quality was poor.

Below, by clicking, you can see the extended fastQC analysis for all samples:

Sample0 Sample1

Sample2 Sample3

**Minion**

This tool was used for searching for the 3' adapter without prior knowledge of the sequence. Minion outputs one or two candidates, accompanied with metadata and selected from a longer list of candidates. If the adapter sequence is known, minion will compare this adapter with all the candidates it found and output the best match.

Unfortunately, one these four samples, minion detects sequences that don’t appear to be adapters. The expected length is supposed to be ~12, and the results is more than this. Probably the results are just some overexpressed sequences that can be found throughout the samples.

After this failed attempt to detect the adapters, Illumina Genome Analyzer was noticed to be used for these samples. Heading over the prior fastQC analysis and examining the “Adapter Content”, it revealed the presence of Illumina Universal Adapters.

**Cutadapt**

Cutadapt was used to find and remove adapter sequences. In this case, as derived from the previous analysis stage, the removal of Illumina Universal Adapter was necessary.

*Illumina Universal Adapter: AGATCGGAAGAG*

**FastQC #2**

A second quality control check was repeated so the changes derived from the cutadapt step can be further inspected.

Although, all the adapters were successfully removed, the existence of zero length sequences was noticed. These can be filtered out the cutadapt stage.

**Bowtie**

The indexes were built from the mouse genome and the alignment process started.

Those are the results from the alignment:

Sample0

# reads processed: 19396915
# reads with at least one reported alignment: 11646200 (60.04%)
# reads that failed to align: 2953663 (15.23%)
# reads with alignments suppressed due to -m: 4797052 (24.73%)
Reported 11646200 alignments to 1 output stream(s)

Sample1

# reads processed: 3572026
# reads with at least one reported alignment: 2728529 (76.39%)
# reads that failed to align: 262188 (7.34%)
# reads with alignments suppressed due to -m: 581309 (16.27%)
Reported 2728529 alignments to 1 output stream(s)

Sample2

# reads processed: 3446127
# reads with at least one reported alignment: 2365739 (68.65%)
# reads that failed to align: 359016 (10.42%)
# reads with alignments suppressed due to -m: 721372 (20.93%)
Reported 2365739 alignments to 1 output stream(s)

Sample3

# reads processed: 22997145
# reads with at least one reported alignment: 4089861 (17.78%)
# reads that failed to align: 13577205 (59.04%)
# reads with alignments suppressed due to -m: 5330079 (23.18%)
Reported 4089861 alignments to 1 output stream(s)

Finally, the sam file was converted to bam, then sorted and finally indexed so it can be loaded into IGV and be used on MACS as well.

**MACS**

The peaks.bed file was then sorted based on their score and the top three were selected.

See, for every sample, the top regions:

Sample0

| chr | start | end | length | -10*log10(pvalue) |
|---|---|---|---|---|
| chr12 | 112996046 | 112998500 | 2455 | 3118.2 |
| chr17 | 25251616 | 25253639 | 2024 | 3100 |
| chr9 | 35112724 | 35113245 | 522 | 3100 |

Sample1

| chr | start | end | length | -10*log10(pvalue) |
|---|---|---|---|---|
| chr17 | 39979830 | 39983741 | 3912 | 3100 |
| chr11 | 108872874 | 108873447 | 574 | 2998.72 |
| chr5 | 147072479 | 147073018 | 540 | 1664.64 |

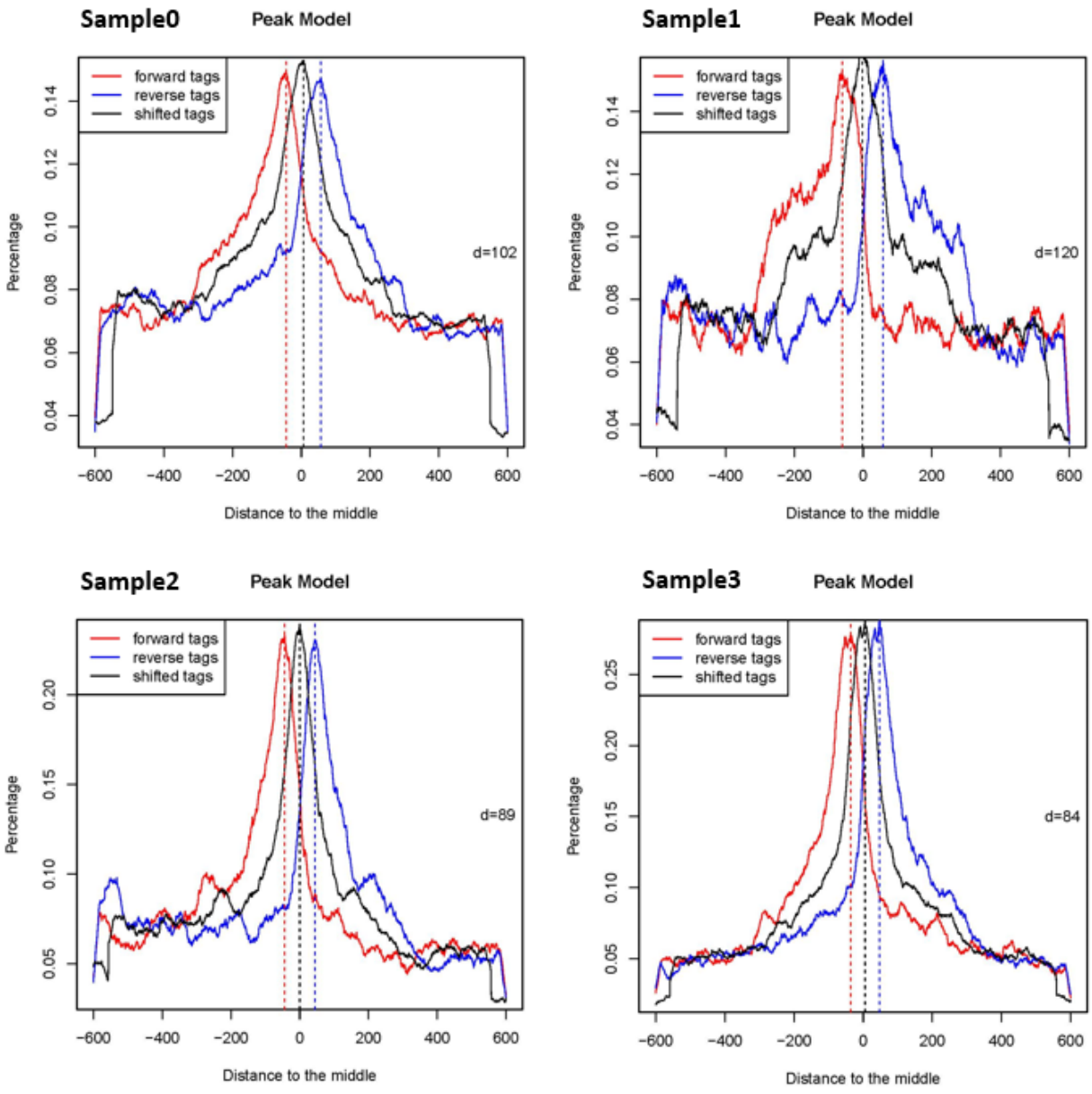
Sample2

| chr | start | end | length | -10*log10(pvalue) |
|---|---|---|---|---|
| chr13 | 21869842 | 21872771 | 2930 | 3184.03 |
| chr13 | 23646580 | 23648688 | 2109 | 3100 |
| chr13 | 23712010 | 23714673 | 2664 | 3100 |

Sample3

| chr | start | end | length | -10*log10(pvalue) |
|---|---|---|---|---|
| chr9 | 35112744 | 35113179 | 436 | 2475.71 |
| chrUn_random | 5495488 | 5497315 | 1828 | 1819.11 |
| chr13 | 23830529 | 23832826 | 2298 | 1462.54 |

The peak model for each sample:

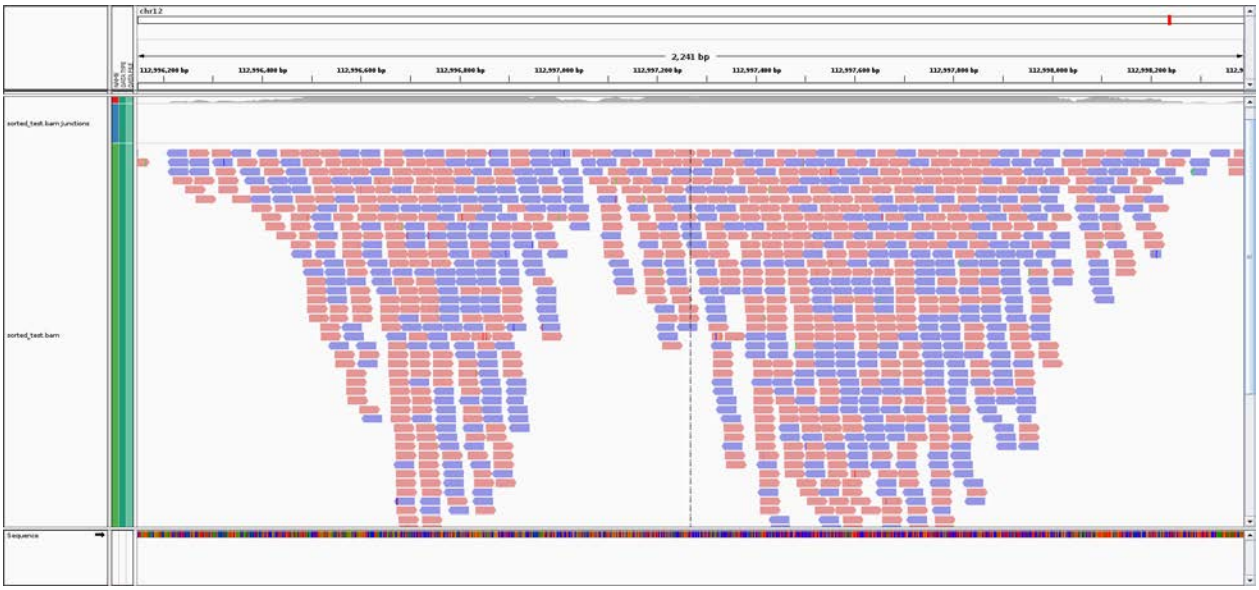


**IGV**

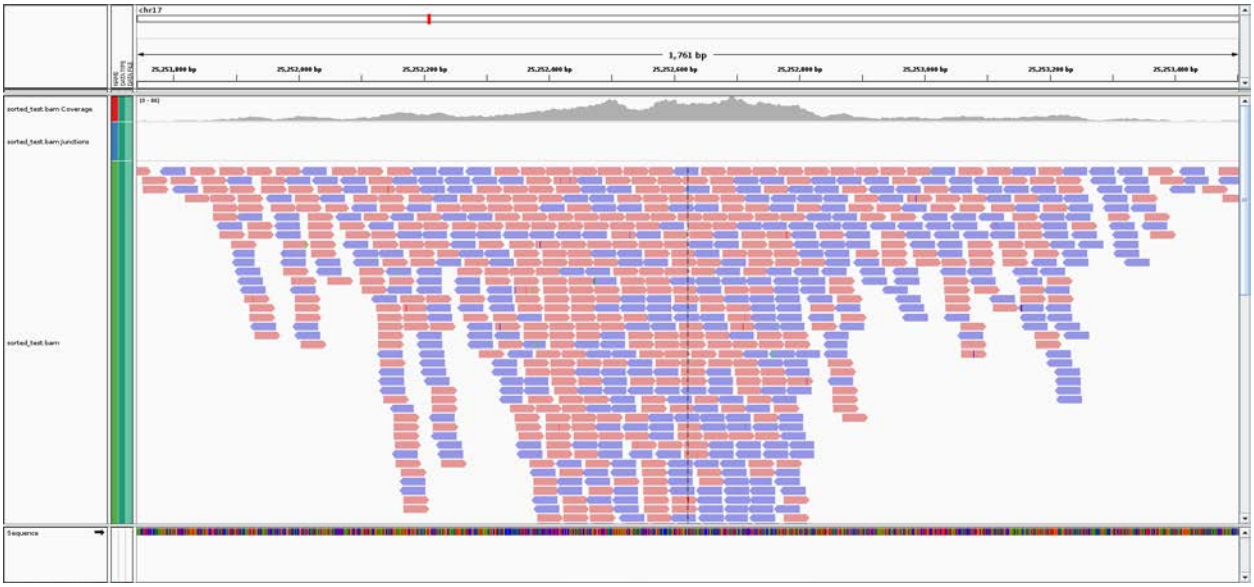
The mouse genome was, firstly loaded, and afterwards each sorted bam file from each sample.

Sample0

-Peak1




-Peak2

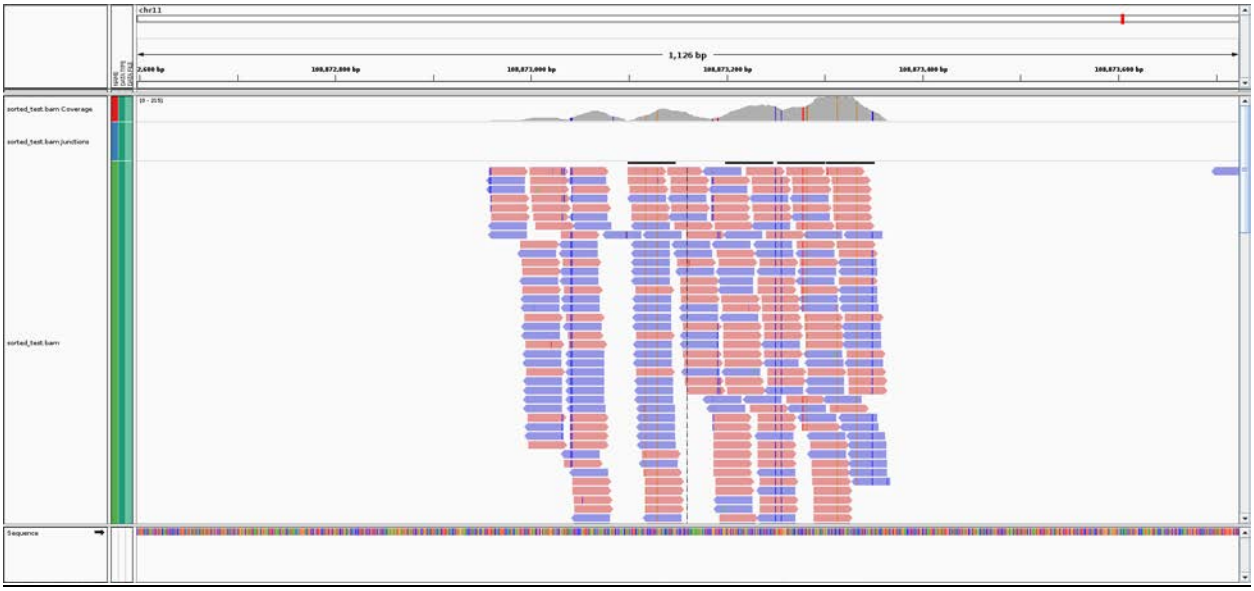


-Peak3

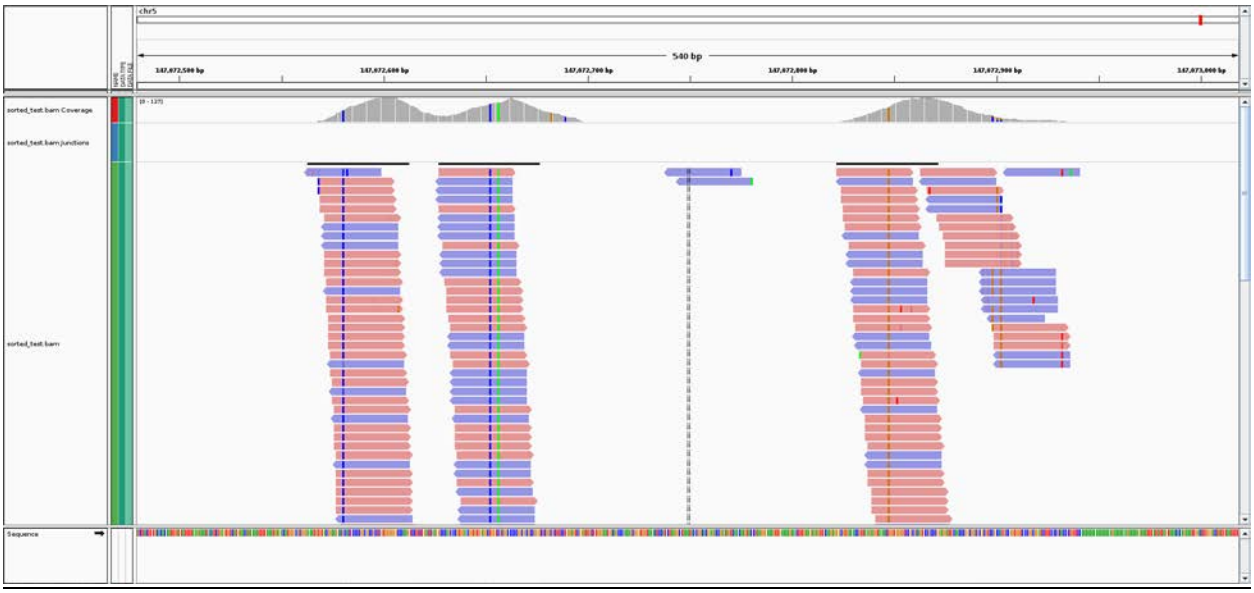

Sample1

-Peak1

-Peak2


chr11
1,126 bp
sorted_test.bam Coverage
sorted_test.bam Junctions
sorted_test.bam
Sequence

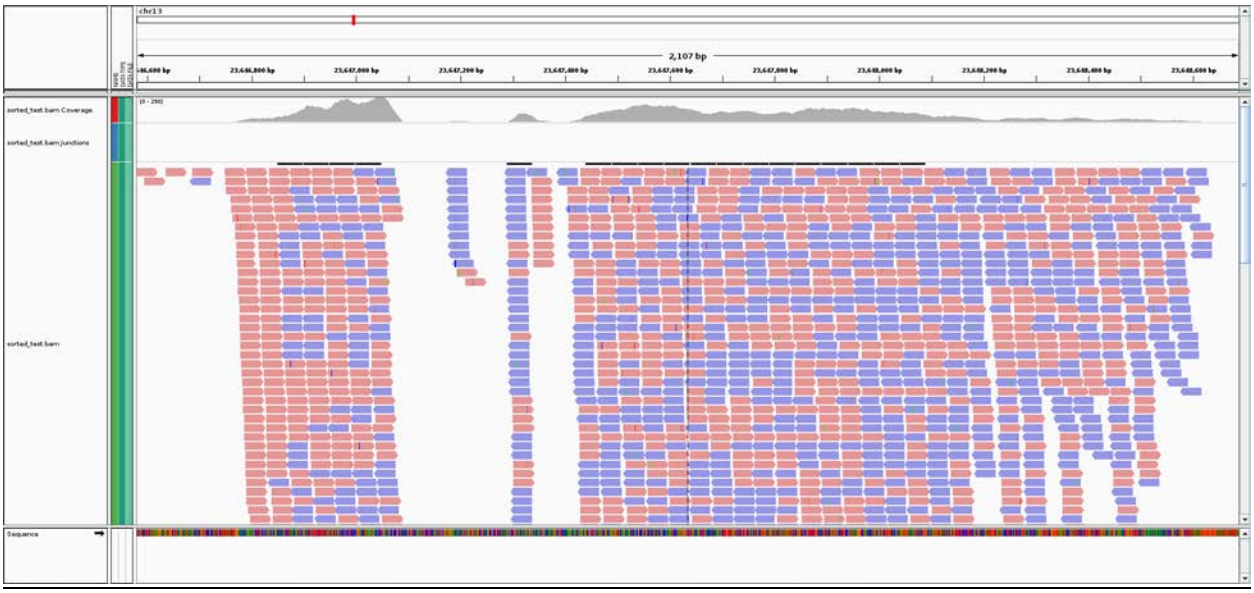
-Peak3


chr5
540 bp
sorted_test.bam Coverage
sorted_test.bam Junctions
sorted_test.bam
Sequence

Sample2

-Peak1

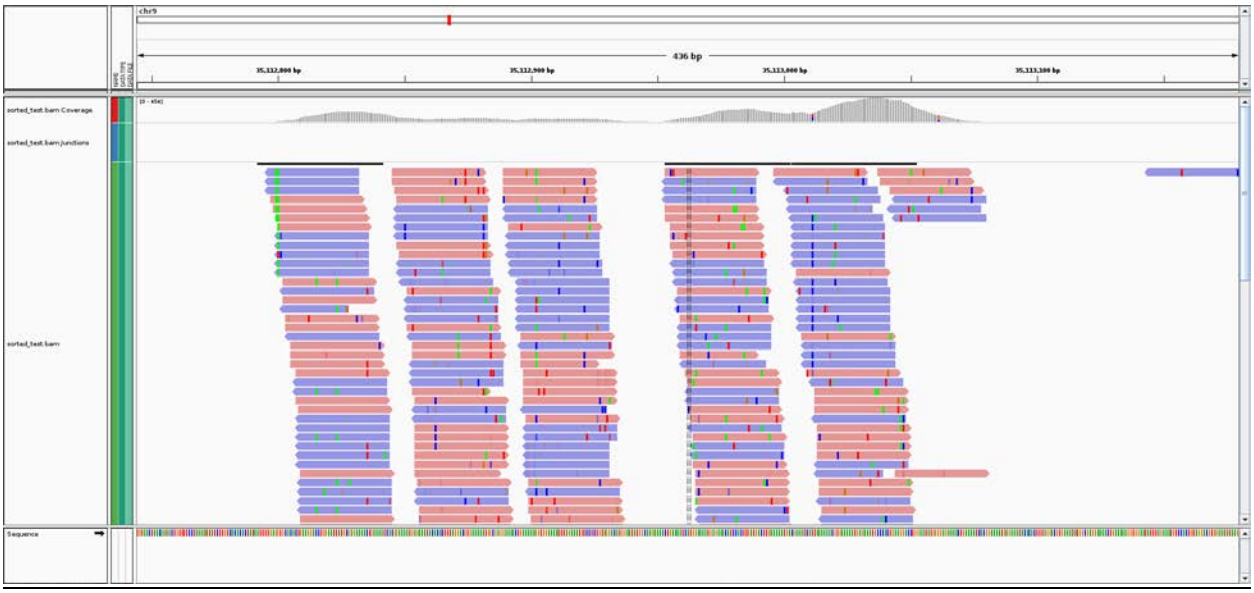

-Peak2



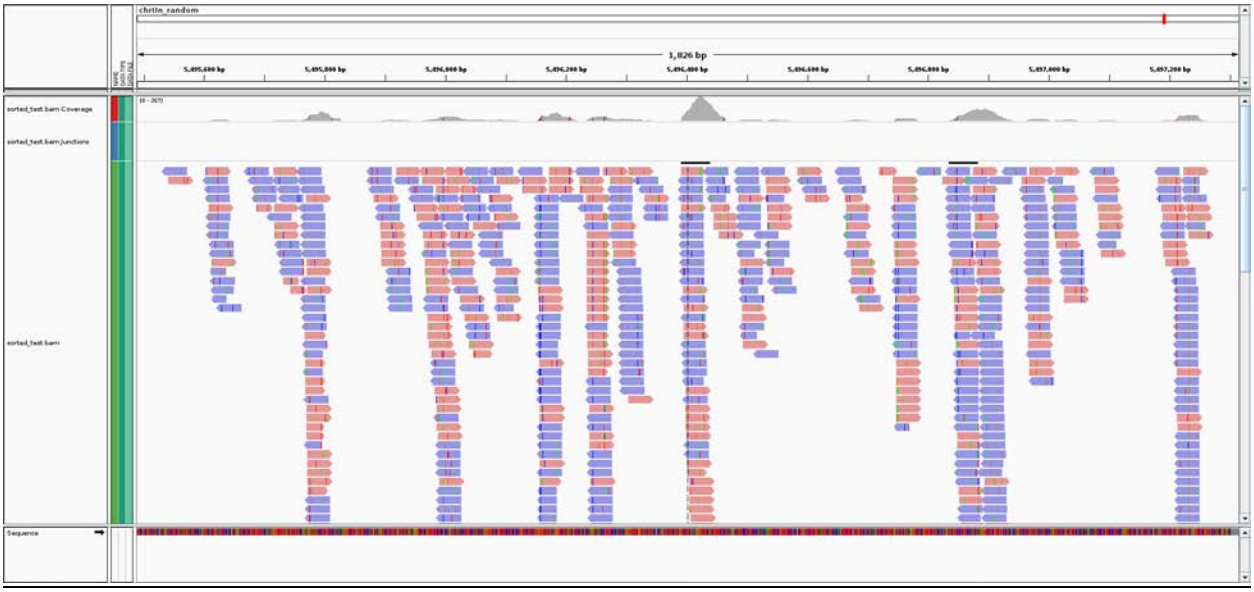
-Peak3


Sample3

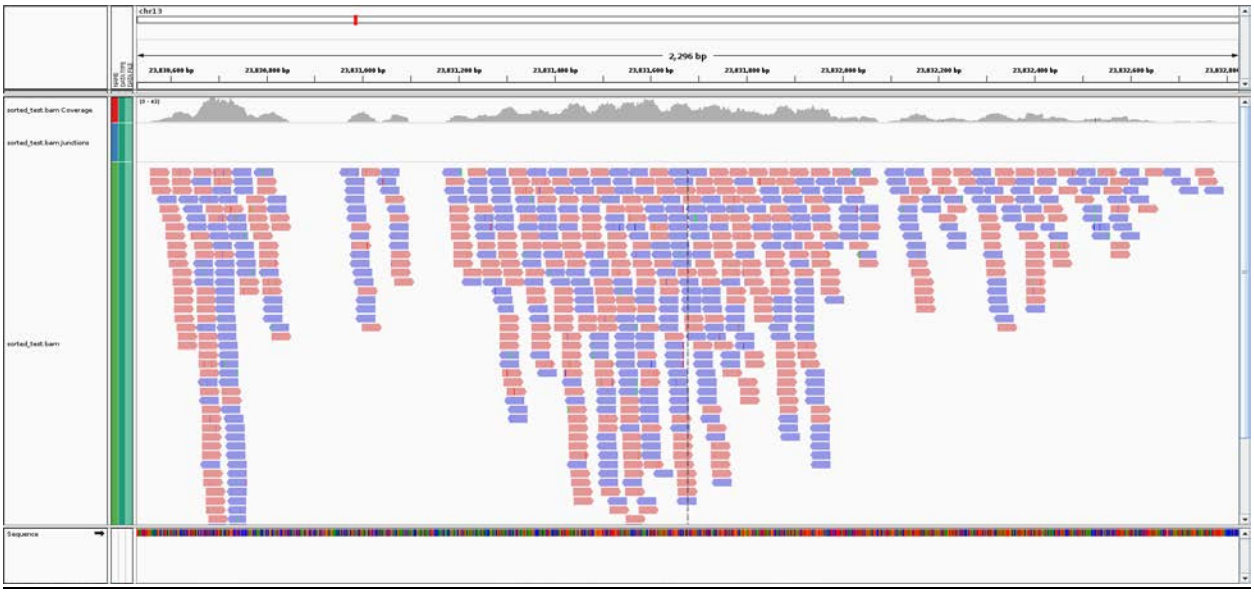
-Peak1



-Peak2


chr1b_random
1,826 bp
sorted_test.bam Coverage
sorted_test.bam Junctions
sorted_test.bam
Sequence

-Peak3


chr1.3
2,296 bp
sorted_test.bam Coverage
sorted_test.bam Junctions
sorted_test.bam
Sequence

**MEME**

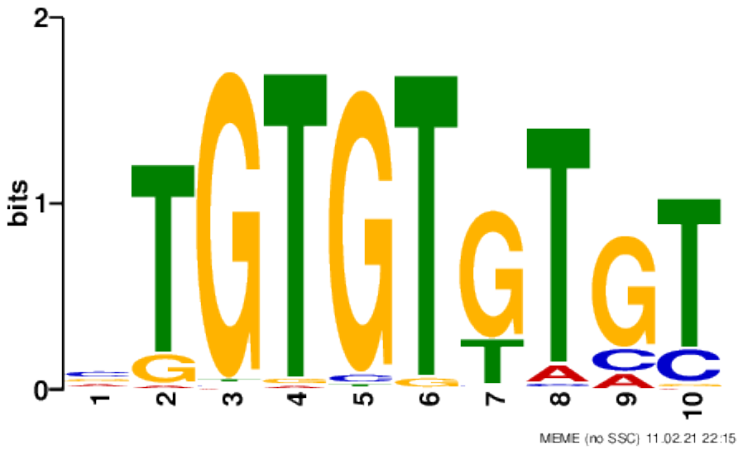
MEME discovers novel, ungapped motifs (recurring, fixed-length patterns) in the sequences and splits variable-length patterns into two or more separate motifs.

A motif is an approximate sequence pattern that occurs repeatedly in a group of related sequences. MEME represents motifs as position-dependent letter-probability matrices that describe the probability of each possible letter at each position in the pattern. Individual MEME motifs do not contain gaps. Patterns with variable-length gaps are split by MEME into two or more separate motifs.

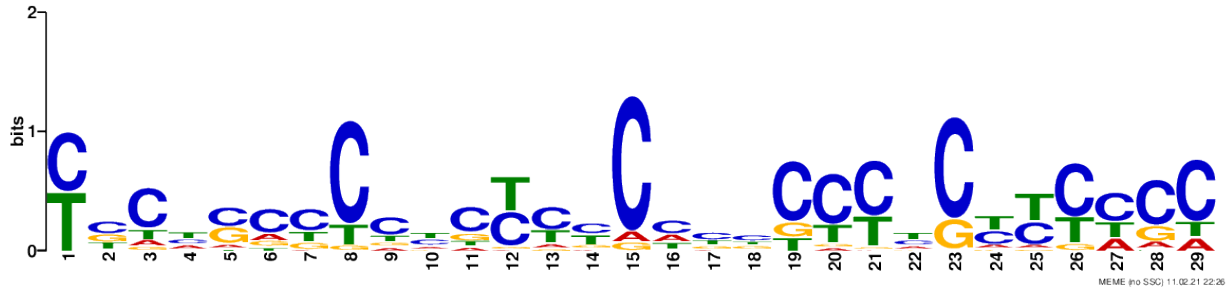
MEME uses statistical modeling techniques to automatically choose the best width, number of occurrences, and description for each motif.

These are the discovered motifs for each sample:

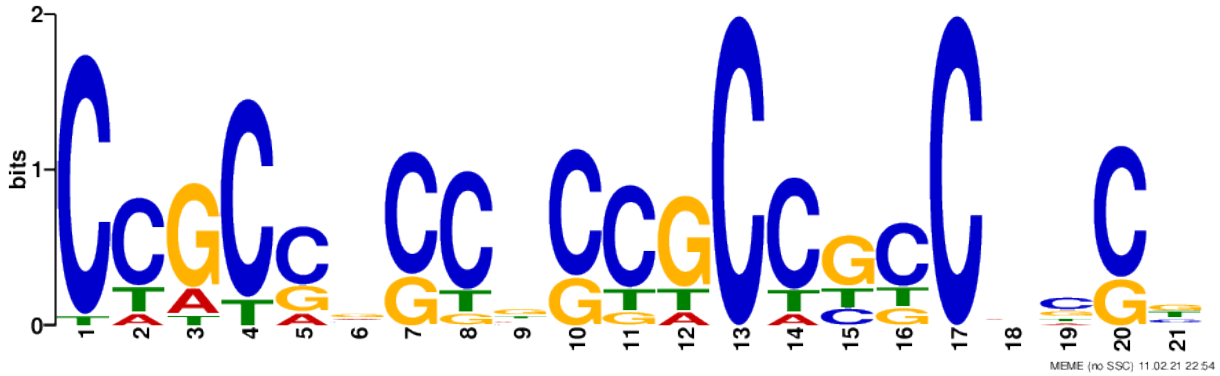
Sample0



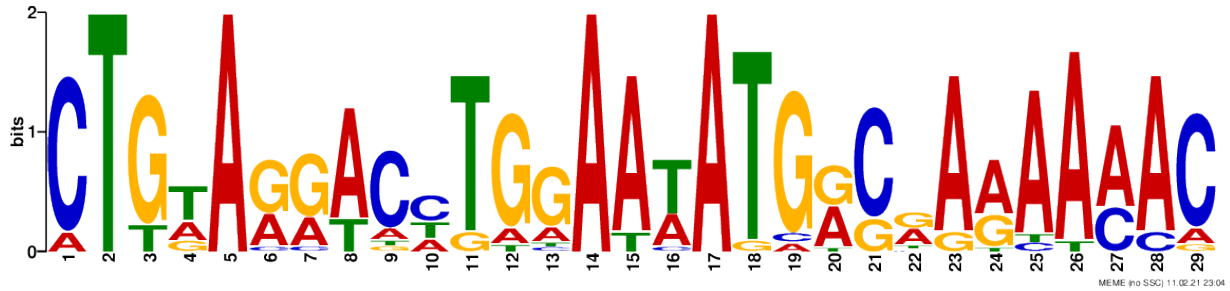
Sample1




Sample2




Sample3



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