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Motif analysis

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SciLifeLab (Long-term Bioinformatics Support)

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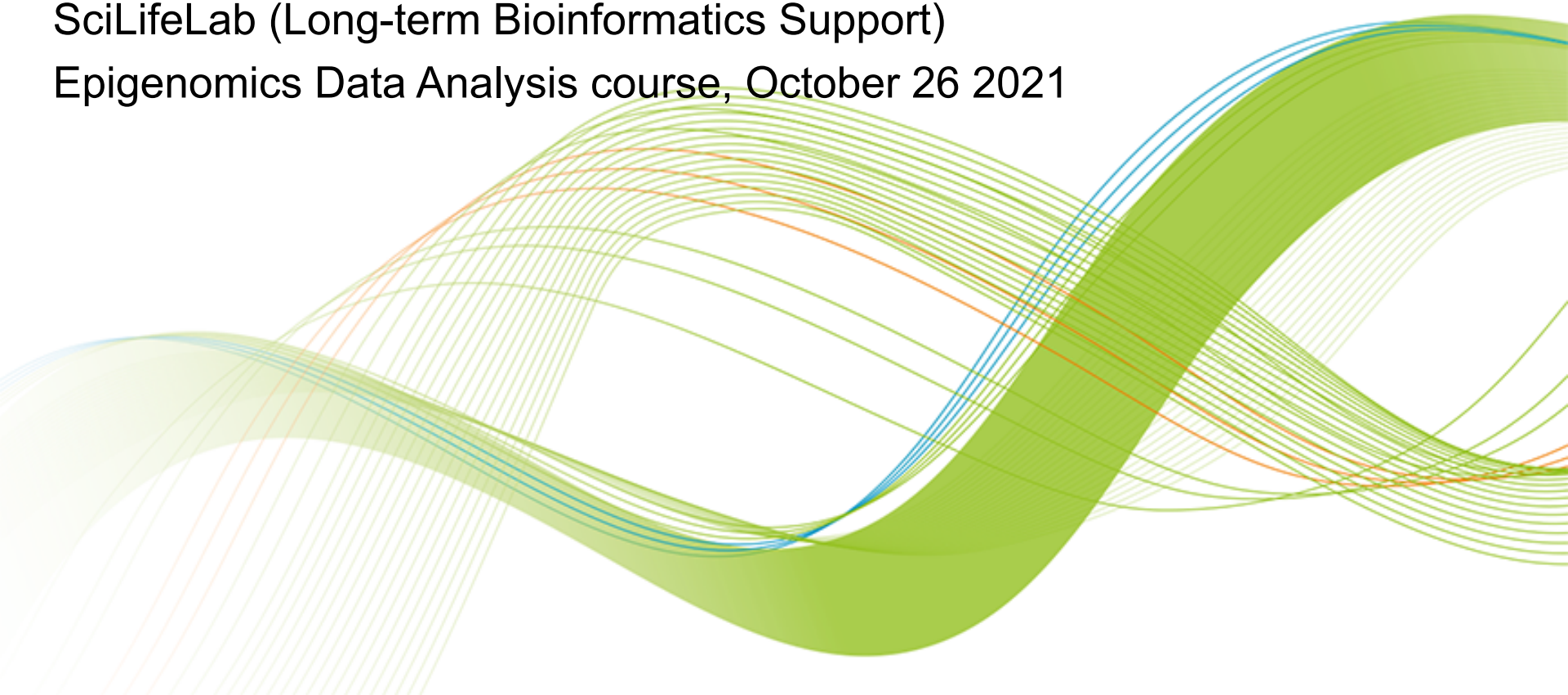


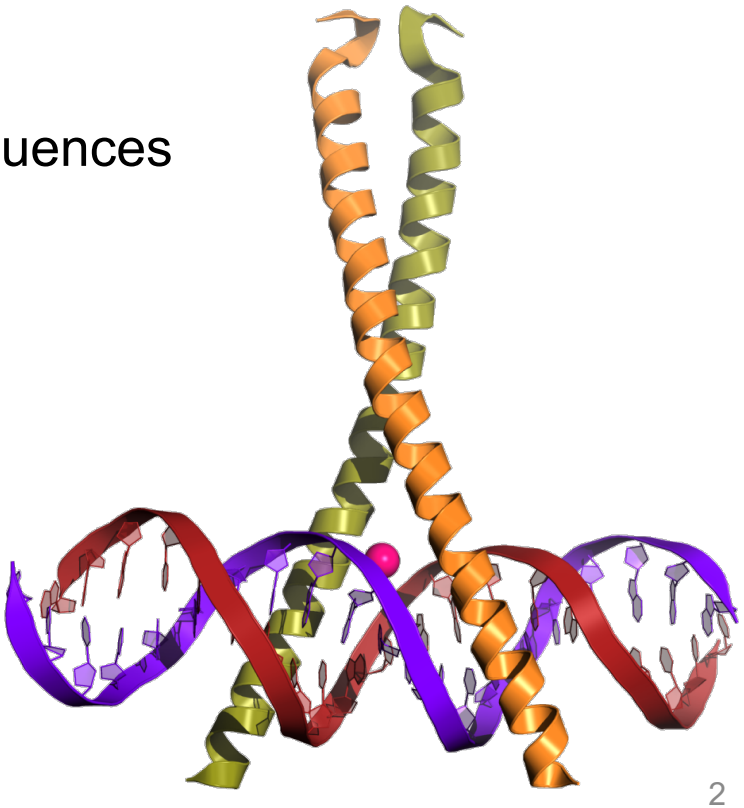
The problem

From a transcription factor (TF) ChIP-seq experiment, find the DNA sequences recognized by the TF.

From a set of open chromatin regions, from an ATAC-seq experiment, find candidate TF binding sites.

In this context: Motif = a set of nucleotide sequences

Typically 4-20 bp



This talk

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- What is a motif? How is it represented?
- *De-novo* motif discovery: What the problem is, principles behind the programs
- Examples of motif discovery programs
- Practical considerations: data size, how to handle repeats etc.

How DNA sequence motifs be represented

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1. As a *sequence* of nucleotides, e.g. CTGGA
2. As a *regular expression*, taking into account ambiguity e.g. [C or G][C or T]GG[G or A]
3. As a *matrix,* based on nucleotide frequency in each position

Pos	1	2	3	4	5
A	0	1	0	0	5
C	5	4	0	0	0
G	4	0	10	10	4
T	1	5	0	0	1

4. More complicated representations, taking dependencies between positions into account (HMMs, dinucleotide matrices, deep learning networks etc.)

Position weight matrices

- A position weight matrix (PWM) is based on nucleotide frequencies in a set of aligned sequences.
- The frequencies are converted to probabilities, and then to log-likelihoods given a background model.

Pos	1	2	3	4	5
A	0	1	0	0	5
C	5	4	0	0	0
G	4	0	10	10	4
T	1	5	0	0	1

Position *frequency* matrix

count nucleotides in each position

Pos	1	2	3	4	5
A	0.0	0.1	0.0	0.0	0.5
C	0.5	0.4	0.0	0.0	0.0
G	0.4	0.0	1.0	1.0	0.4
T	0.1	0.5	0.0	0.0	0.1

Position *probability* matrix

divide by total nr of sequences

Pos	1	2	3	4	5
A	-Inf	-1.32	-Inf	-Inf	1.0
C	1.0	0.68	-Inf	-Inf	-Inf
G	0.68	-Inf	2.0	2.0	0.68
T	-1.32	1.0	-Inf	-Inf	-1.32

Position *weight* matrix

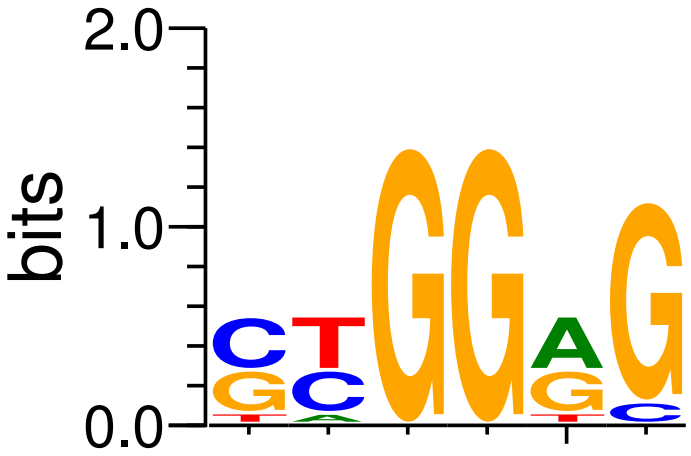
divide by background freq, and log-transform $-\log(m_{n,p}/b_n)$

- We might need to add a pseudo count to the frequency matrix, to avoid –Inf.

Sequence logos

- Sequence logos are used to visualize PWMs.
- Nucleotide frequency and information content for each position can be represented.

Pos	1	2	3	4	5
A	0	1	0	0	0
C	4	4	0	0	5
G	5	5	10	10	4
T	1	0	0	0	1



Height: 2 – entropy $= 2 - \sum_{i=1}^{n} \mathrm{P}(x_i) \log_b \mathrm{P}(x_i)$

Databases with TF binding site motifs

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- JASPAR (http://jaspar.genereg.net). Good, curated, free, data base with around 1500 motifs from all kinds of species.
- Transfac (http://genexplain.com/transfac/). Good, curated, not free, data base with around 2800 motifs from all kinds of species.
  - Older version is free for academic use.
- Other databases
  - HOCOMOCO (human only) http://hocomoco11.autosome.ru
  - footprintDB (combining several databases) http://floresta.eead.csic.es/footprintdb/index.php

Scanning the genome with a PWM

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- Every sequence can be scored on how well it matches the PWM, by adding up the scores for each position:

Pos	1	2	3	4	5
A	-Inf	-1.32	-Inf	-Inf	1.0
C	1.0	0.68	-Inf	-Inf	-Inf
G	0.68	-Inf	2.0	2.0	0.68
T	-1.32	1.0	-Inf	-Inf	-1.32

GAGGG → 0.68 -1.32 + 2.0 +2.0 + 0.68 = 4.04
CTGGG → 1.0 + 1.0 + 2.0 + 2.0 + 1.0 = 7
CTGAG → 1.0 + 1.0 - Inf + 2.0 + 1.0 = - Inf

- The score represents the log likelihood of the sequence being a motif compared to bg
- High scores → likely strong TF binding → long time spent on DNA by TF
- Useful to have a cutoff on what we consider is a match. Setting cutoff can be tricky!

Limitations of position weight matrices

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- In 90% of tested cases, matrix based models perform as well as more complex models (Weirauch et al. Nature Biotech. 2013).
- But PWMs can be inaccurate if there is
  - Dependencies between nucleotides
  - Variable spacing between sequences

De-novo motif finding

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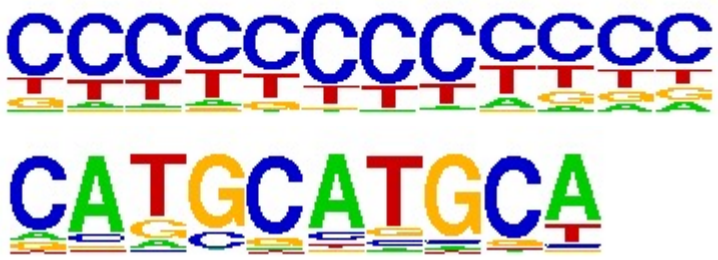
- Given a set of transcription factor binding sites (e.g. from ChIP-seq), are any motifs enriched?
- Some kind of background model is needed
  - A set of background sequences
    - Regions nearby the peaks (e.g. 2 Kbp away), with similar GC content
  - Nucleotide (or dinucleotide) frequencies
  - A bad background model will give strange and misleading results!



Motif finding methods

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- We need methods to search the space of possible motifs
- We also need a way to score motif candidates (e.g. enrichment, complexity)
- Optimal results are not guaranteed.

MEME

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- Method:
  - Starts with a guess, M, of what the motif might be. It then produces estimates, L, of where motif is located.
  - Given L, the motif M is updated. Then L is updated with a new motif and so on, until the motif M doesn't change much.
  - When the motif search has converged, the resulting motif is scored (based on enrichment and information content).
  - To find more motifs, all occurrences of the motif are then removed from the input sequences, and the algortim is the re-run with a new start guess.
- Output
  - A set of PWMs, with scores and p-values
- Pros: Old, widely used method. Often works well.
- Cons: Slow, has trouble handling large inputs (>500 peaks)

MEME

Multiple Em for Motif Elicitation

DREME

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- Method:
  - Look at all 3-8mers to find the most enriched sequences (Fisher test)
  - Iteratively, try to make these more general with search
    - CTGGG
    - → CTGG[G or A]
    - → C[C or T]GG[G or A]
    - → [C or G][C or T]GG[G or A]
  - Convert this to PWM
- Output: PWMs, with p-values
- Pros: Very fast, good performance
- Cons: Restricted to short sequences (up to 8 bp). Does not take nucleotide frequency into account.

(Bailey, Bioinformatics 2011)

DREME

Discriminative Regular Expression Motif Elicitation

Homer

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- Method
  - Looks at all 8,10 and 12-mers to find the most enriched.
  - The most enriched sequences are then converted to weight matrices are refined.
- Output
  - A set of PWMs, with info on e-values and which known motif it's similar to.
  - If any known motifs are enriched in the given regions.
- Pros
  - Nice output, includes matching to known motifs
  - Quite fast
  - Usually works well
- Cons
  - The documentation is not good
  - It's a bit hard to install, need to install genomes too.

Practical considerations

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- Less information content → harder problem
  - Short motifs are harder to find
  - Degenerate motifs are harder to find
- Which peaks to use?
  - Some methods will have problems handling tens of thousands of peaks.
  - Also, many weak peaks don't provide useful information
  - → often only the top 500 etc. peaks are used.
- Repeats (e.g. low complexity repeats) can throw the motif finding methods off. → Work on repeat masked sequences!

How well do these methods work?

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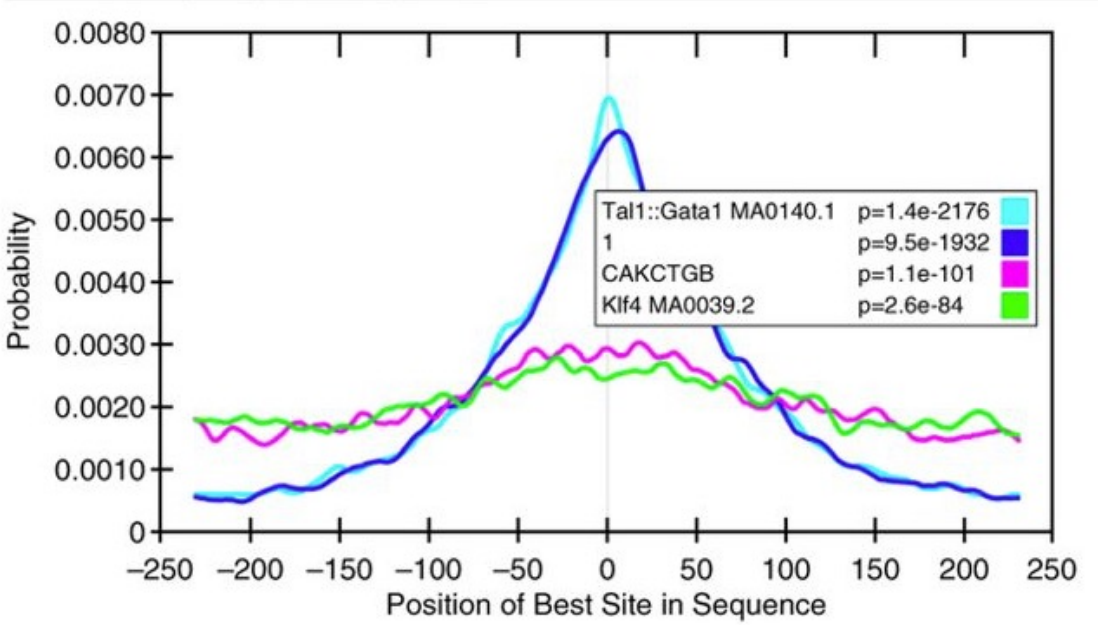
- There is no good benchmarking study on motif finding in ChIP-seq data, but usually finding the main motif is not that difficult
  - ChIP-seq gives short regions to look in
  - The top ChIP-seq peaks are typically very enriched for the motif of interest.
- There might also be co-factor motifs. These are harder to find.
- Compare this to analysis of promoters of co-regulated genes:
  - We have very long promoters to search for motifs
  - We have don’t have as clear enrichment of the motifs.

Further analysis

- PhyloGibbs – incorporating sequence conservation in the motif finding.
- Ensemble methods – combining the results from several motif finding programs
- TomTom – Comparison of a new motif to a database of known motifs
- Centrimo – Motif location.



Exercise

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- Takes sets of peaks from ENCODE
  - ChIP-seq against CTCF (human and mouse data sets)
  - ChIP-seq against REST, from previous lab
- Try a few different motif finders
  - DREME
  - MEME
  - HOMER
- Try a motif comparison tool, TomTom