Course organization

- Introduction (Week 1-2)
  - Course introduction
  - A brief introduction to molecular biology
  - A brief introduction to sequence comparison
- Part I: Algorithms for Sequence Analysis (Week 3 - 11)
  - Chapter 1-3, Models and theories
    - » Probability theory and Statistics (Week 4)
    - » Algorithm complexity analysis (Week 5)
    - » Classic algorithms (Week 6)
    - » Lab: Linux and Perl
  - Chapter 4, Sequence alignment (week 7)
  - Chapter 5, Hidden Markov Models (week 8)
  - Chapter 6. Multiple sequence alignment (week 10)
  - **Chapter 7. Motif finding (week 11)**
  - **Chapter 8. Sequence binning (week 11)**
- Part II: Algorithms for Network Biology (Week 12 - 16)

Chapter 7
Motif Finding

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Contents

1. Reading materials
2. Motif finding
    - Motif
    - WMM
    - Motif finding methods

Reading materials

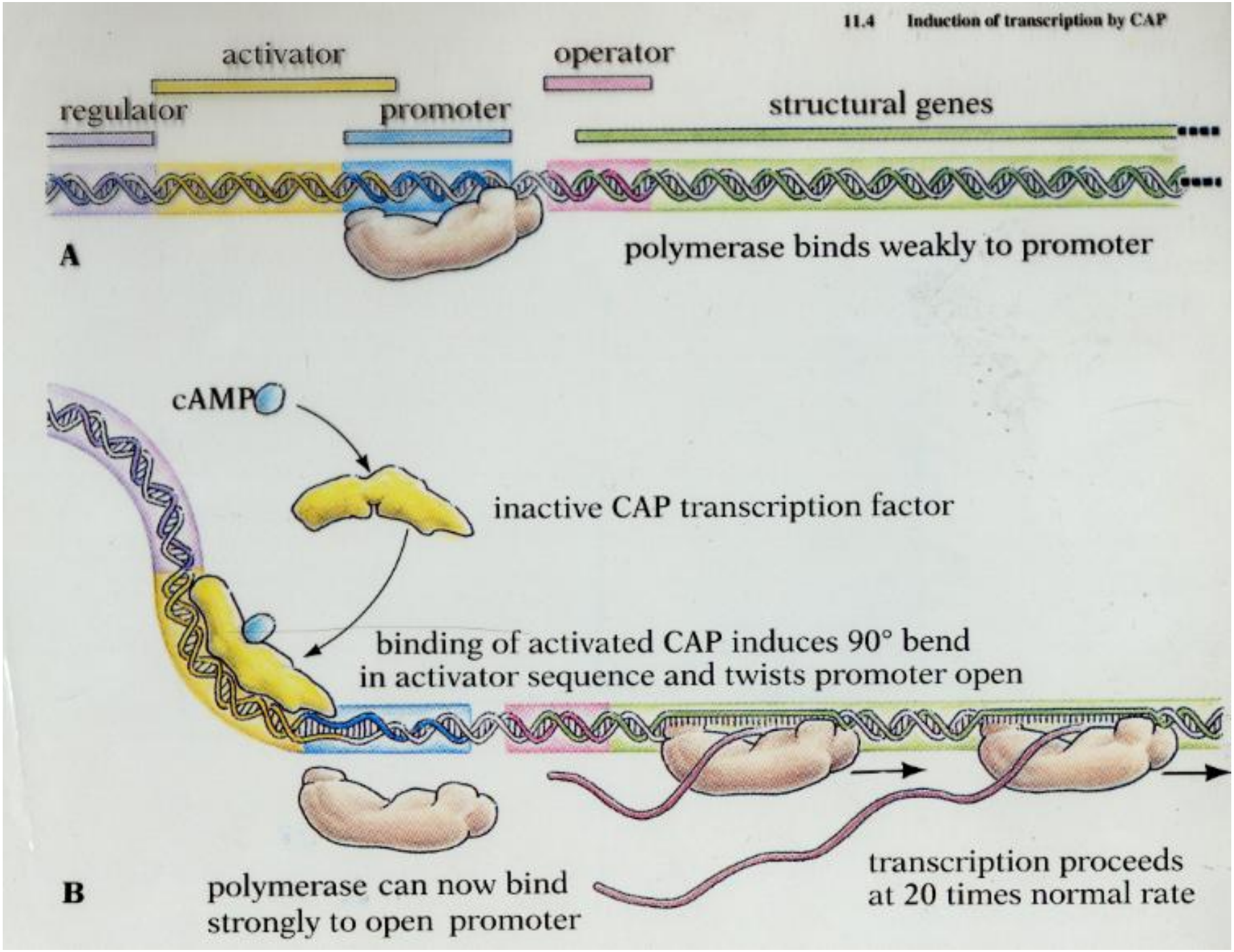
- Tompa et al (2005), “Assessing computational tools for the discovery of transcription factor binding sites”, Nature Biotechnology, 23(2):137-144

Sequence Motifs

- Motif: subsequence with some specific function
- May be in DNA, RNA, protein
- Function may be context dependent
  - Ribosome binding site must be transcribed
  - RNA, protein motifs may depend on structure
  - May be gapped or ungapped
- Use model to search for (predict) new sites
  - Models may be simple sequences (regular expressions) or probabilistic patterns
- Modeling approach depends on data available
  - Quantitative/qualitative

Motifs: DNA binding sites

- Gene regulation often controlled by proteins (transcription factors) that bind to specific DNA sequences to activate or repress transcription
- Binding sites are usually short (6-30bp) and typically ungapped, probabilistic patterns
- Would like to have a quantitative model that predicts binding affinity and/or occupancy


11.4 Induction of transcription by CAP
activator
operator
regulator
promoter
structural genes
A
polymerase binds weakly to promoter
cAMP
inactive CAP transcription factor
binding of activated CAP induces 90° bend
in activator sequence and twists promoter open
B
polymerase can now bind
strongly to open promoter
transcription proceeds
at 20 times normal rate

Weight Matrix Model

A:	-8	10	-1	2	1	-8
C:	-10	-9	-3	-2	-1	-12
G:	-7	-9	-1	-1	-4	-9
T:	10	-6	9	0	-1	11

Weight Matrix Model

-24

….A **C** **T** **A** **T** **A** **A** T G T.

A:	-8	10	**-1**	2	**1**	**-8**
C:	**-10**	-9	-3	-2	-1	-12
G:	-7	-9	-1	-1	-4	-9
T:	**10**	**-6**	9	**0**	-1	11

Weight Matrix Model

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….A C **T A T A A T** G T…

A:	-8	**10**	-1	**2**	**1**	-8
C:	-10	-9	-3	-2	-1	-12
G:	-7	-9	-1	-1	-4	-9
T:	**10**	-6	**9**	0	-1	**11**

How to get optimal models

- Quantitative binding data
  - Can do regression to get best fit
  - Can test different models
    - Must take into account that a more complex model can always give better fit, but is it useful?
- Qualitative binding data – set of sites
  - Log-odds methods
  - Can use minimum volume method (QP)

A.

A	9	214	63	142	118	8
C	22	7	26	31	52	13
G	18	2	29	38	29	5
T	193	19	124	31	43	216

N(b,i)

B.

A	0.04	0.88	0.26	0.59	0.49	0.03
C	0.09	0.03	0.11	0.13	0.22	0.05
G	0.07	0.01	0.12	0.16	0.12	0.02
T	0.80	0.08	0.51	0.13	0.18	0.89

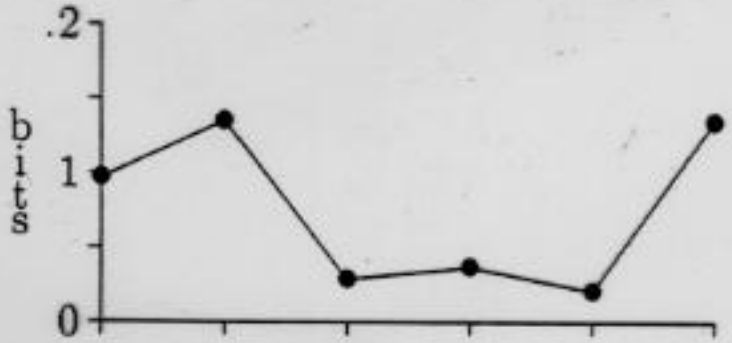
F(b,i)

C.

A	-2.76	1.82	0.06	1.23	0.96	-2.92
C	-1.46	-3.11	-1.22	-1.00	-0.22	-2.21
G	-1.76	-5.00	-1.06	-0.67	-1.06	-3.58
T	1.67	-1.66	1.04	-1.00	-0.49	1.84

$S(b,i) = \log[F(b,i)/P(b)]$

D.



$I(i) = \sum_{b} F(b,i)S(b,i)$

Likelihood Ratio Statistics Primer

Given two probability distributions P_i *and* Q_I

$$\sum P_i = \sum Q_i = 1$$

And some data, D_i, which is number of times each type *i* is observed in *N* total observations

The Likelihood Ratio of the data being from distribution Q_i versus P_i is:

$$\mathbf{LR} = \prod (Q_i/P_i)^{Di}$$

And the log-Likelihood Ratio is

$$\mathbf{LLR} = \sum D_i \ln (Q_i/P_i)$$

LLR = $\sum D_i \ln (Q_i/P_i)$

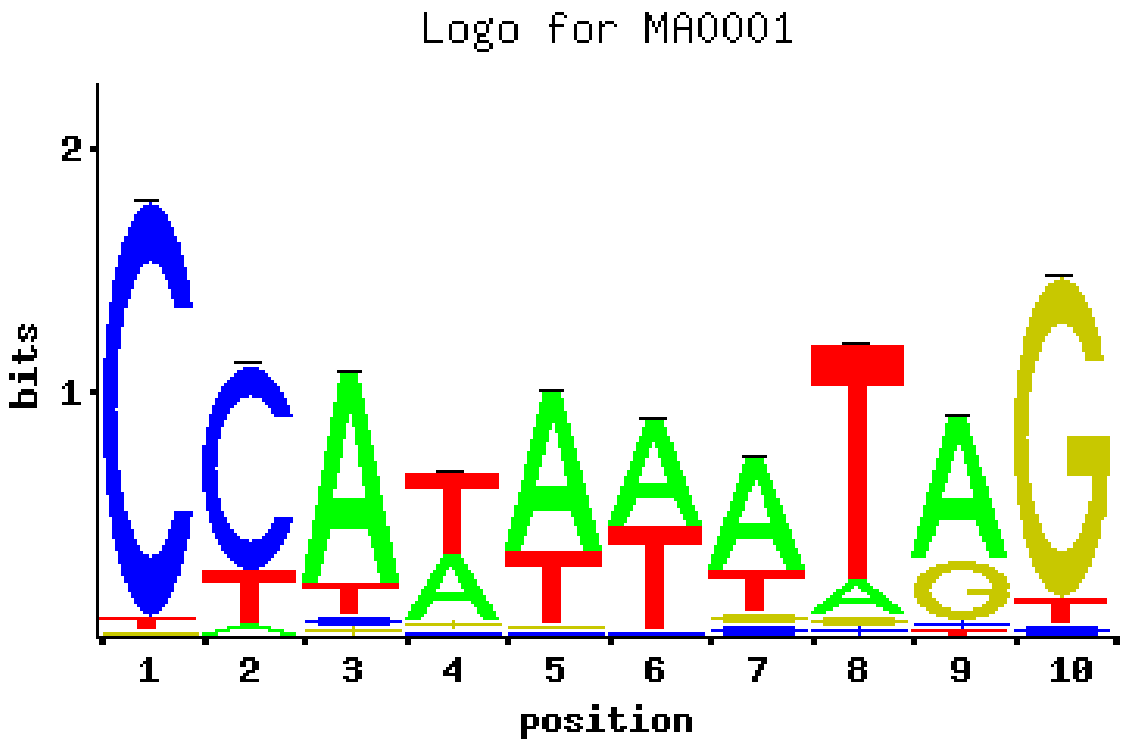
Maximum likelihood distribution is $Q_i = D_i/N$

So max **LLR** = $N \sum Q_i \ln (Q_i/P_i)$

$\sum Q_i \ln (Q_i/P_i) \geq 0$

≡ Information Content
Relative Entropy
Kullbach-Liebler Distance

Related to G-statistic and χ^2



A	0	3	79	40	66	48	65	11	65	0
C	94	75	4	3	1	2	5	2	3	3
G	1	0	3	4	1	0	5	3	28	88
T	2	19	11	50	29	47	22	81	1	6

Motif Discovery

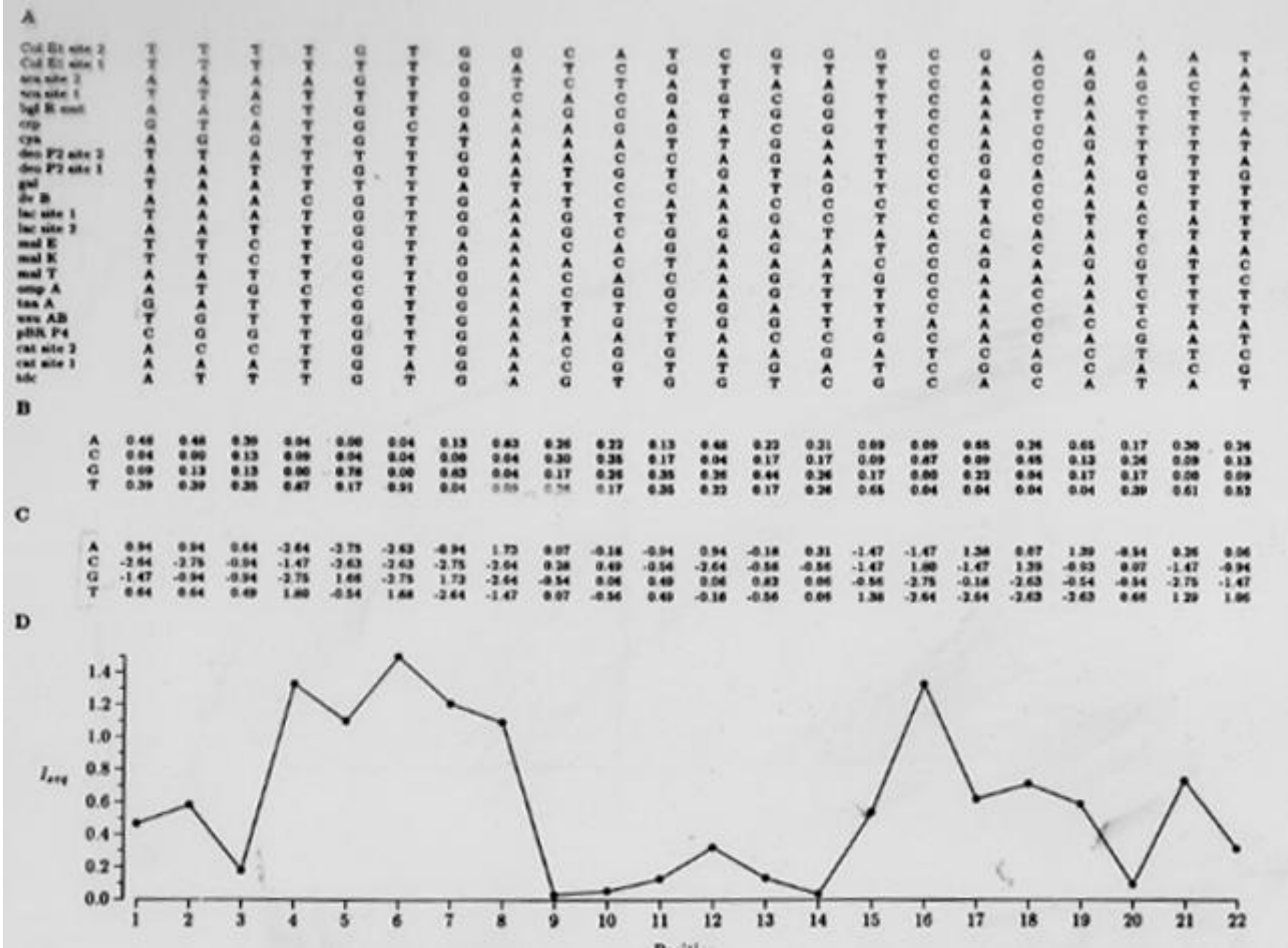
Find significant motifs in long sequences

- Types of data
  - Co-regulated promoters
  - Segments bound to specific proteins
  - Phylogenetically conserved segments
- Algorithm classes – search space
  - Pattern searches – consensus motifs
  - Alignment searches – PWM motifs
- Objective Functions

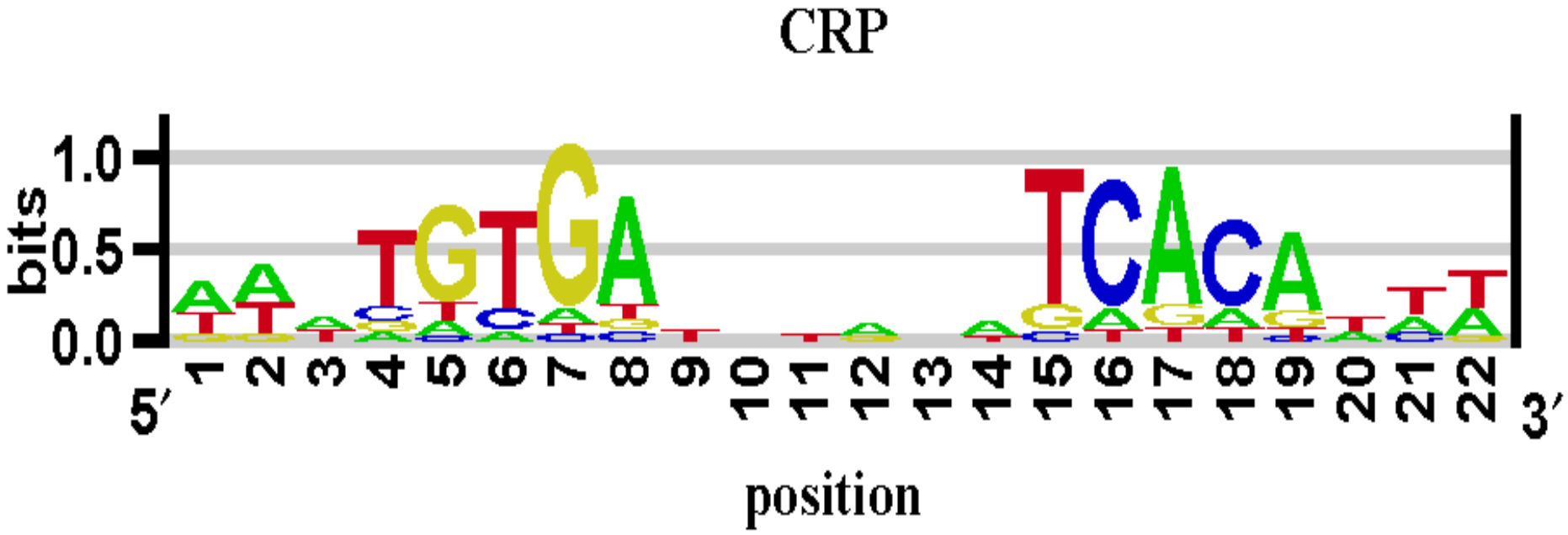
Example (small) dataset

```
CE1CG
\TAATGTTTGTGCTGGTTTTTGTGGCATCGGGCGAGAATAGCGCGTGGTGTGAAAGACTGTTTTTTTGATCGTTTTCACAAAAATGGAAGTCCACAGTCTTGACAG\
ECOARABOP
\GACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCATAAG\
ECOBGLR1
\ACAAATCCCAATAACTTAATTATTGGGATTTGTTATATATAACTTTATAAATTCCTAAAATTACACAAAGTTAATAACTGTGAGCATGGTCATATTTTTATCAAT\
ECOCRP
\CACAAAGCGAAAGCTATGCTAAAACAGTCAGGATGCTACAGTAATACATTGATGTACTGCATGTATGCAAAGGACGTCACATTACCGTGCAGTACAGTTGATAGC\
ECOCYA
\ACGGTGCTACACTTGTATGTAGCGCATCTTTCTTTACGGTCAATCAGCAAGGTGTTAAATTGATCACGTTTTAGACCATTTTTTCGTCGTGAAACTAAAAAAACC\
ECODEOP2
\AGTGAATTATTTGAACCAGATCGCATTACAGTGATGCAAACTTGTAAGTAGATTTCCTTAATTGTGATGTGTATCGAAGTGTGTTGCGGAGTAGATGTTAGAATA\
ECOGALE
\GCGCATAAAAAACGGCTAAATTCTTGTGTAAACGATTCCACTAATTTATTCCATGTCACACTTTTCGCATCTTTGTTATGCTATGGTTATTTCATACCATAAGCC\
ECOILVBPR
\GCTCCGGCGGGGTTTTTTGTTATCTGCAATTCAGTACAAAACGTGATCAACCCCTCAATTTTCCCTTTGCTGAAAAATTTTCCATTGTCTCCCCTGTAAAGCTGT\
ECOLAC
\AACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCAC\
ECOMALBA
\ACATTACCGCCAATTCTGTAACAGAGATCACACAAAGCGACGGTGGGGCGTAGGGGCAAGGAGGATGGAAAGAGGTTGCCGTATAAAGAAACTAGAGTCCGTTTA\
ECOMALBA
\GGAGGAGGCGGGAGGATGAGAACACGGCTTCTGTGAACTAAACCGAGGTCATGTAAGGAATTTCGTGATGTTGCTTGCAAAAATCGTGGCGATTTTATGTGCGCA\
ECOMALT
\GATCAGCGTCGTTTTAGGTGAGTTGTTAATAAAGATTTGGAATTGTGACACAGTGCAAATTCAGACACATAAAAAAACGTCATCGCTTGCATTAGAAAGGTTTCT\
ECOOMPA
\GCTGACAAAAAAGATTAAACATACCTTATACAAGACTTTTTTTTCATATGCCTGACGGAGTTCACACTTGTAAGTTTTCAACTACGTTGTAGACTTTACATCGCC\
ECOTNAA
\TTTTTTAAACATTAAAATTCTTACGTAATTTATAATCTTTAAAAAAAGCATTTAATATTGCTCCCCGAACGATTGTGATTCGATTCACATTTAAACAATTTCAGA\
ECOUXU1
\CCCATGAGAGTGAAATTGTTGTGATGTGGTTAACCCAATTAGAATTCGGGATTGACATGTCTTACCAAAAGGTAGAACTTATACGCCATCTCATCCGATGCAAGC\
PBR322
\CTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTC\
TRN9CAT
\CTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGATCGGCACG\
TDC
\GATTTTTATACTTTAACTTGTTGATATTTAAAGGTATTTAATTGTAATAACGATACTCTGGAAAGTATTGAAAGTTAATTTGTGAGTGGTCGCACATATCCTGTT\
```

Example Output



Example Output



Types of Motifs

1. Motif: Consensus Sequence pattern
   - May include degenerate bases and allow for mismatches
   - *Search space is over possible patterns*

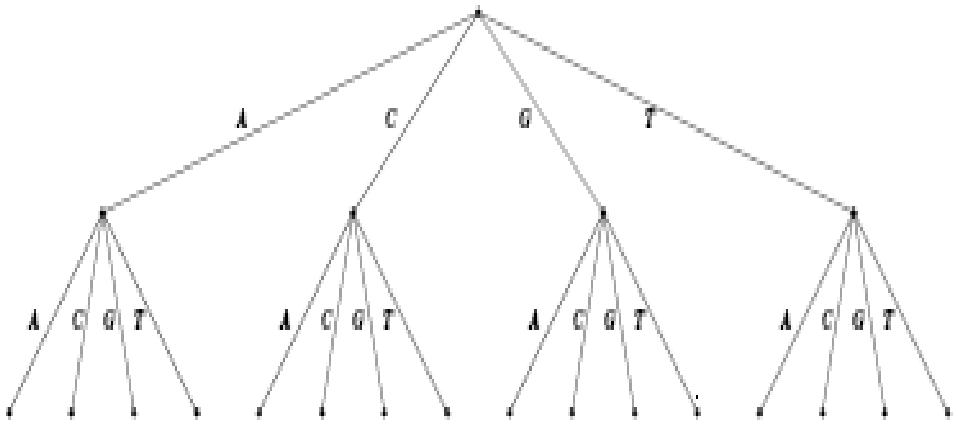
2. Weight Matrix (PWM, Profile, PSSM)
   - Might go to higher order models
   - *Search space is over possible alignments*

Pattern based algorithms

- Motif length l, mismatches m; N seqs, L long
- 4^l patterns, search for most common (or most significant) allowing up to m mismatches
  - P-value from background distribution
  - Can allow for m mismatches
  - Can allow degenerate positions
  - Can just search using existing l-mers

- Can use suffix tree for efficient search of patterns allowing mismatches

Suffix Trees



All sequneces included in O(NL) time and space

Each node can be labeled with N-bit string to indicate which sequences contain specific l-mer

Each pattern can be searched for in linear time, can find all patterns that match criteria of occurring in at least k/N seqs

Significance of each matching Pattern can be found from an Expectation based on background

Can allow m mismatches:
At each string keep track of number of mismatches to pattern being considered and continue down branches until that is exceeded.
Can speed up by requiring the mismatches to be spaced; i.e. not all at beginning of string

WEEDER program: good performance on benchmark tests.

Alignment (Profile) based methods

- Greedy algorithm (Consensus)
- Expectation Maximization (MEME)
- Gibbs Sampler
- Regression (MatrixReduce)

- Can use phylogenetic conservation

Greedy Algorithm (Consensus)

- Simple version: assume every sequence contains at least one true binding site
- Using each l-mer find best match to generate 2-seq alignments
- Using top K PWMs to search remaining sequences to include a new sequence
- Repeat until all seqs contribute
  - Or objective function is maximized (IC, p-value)

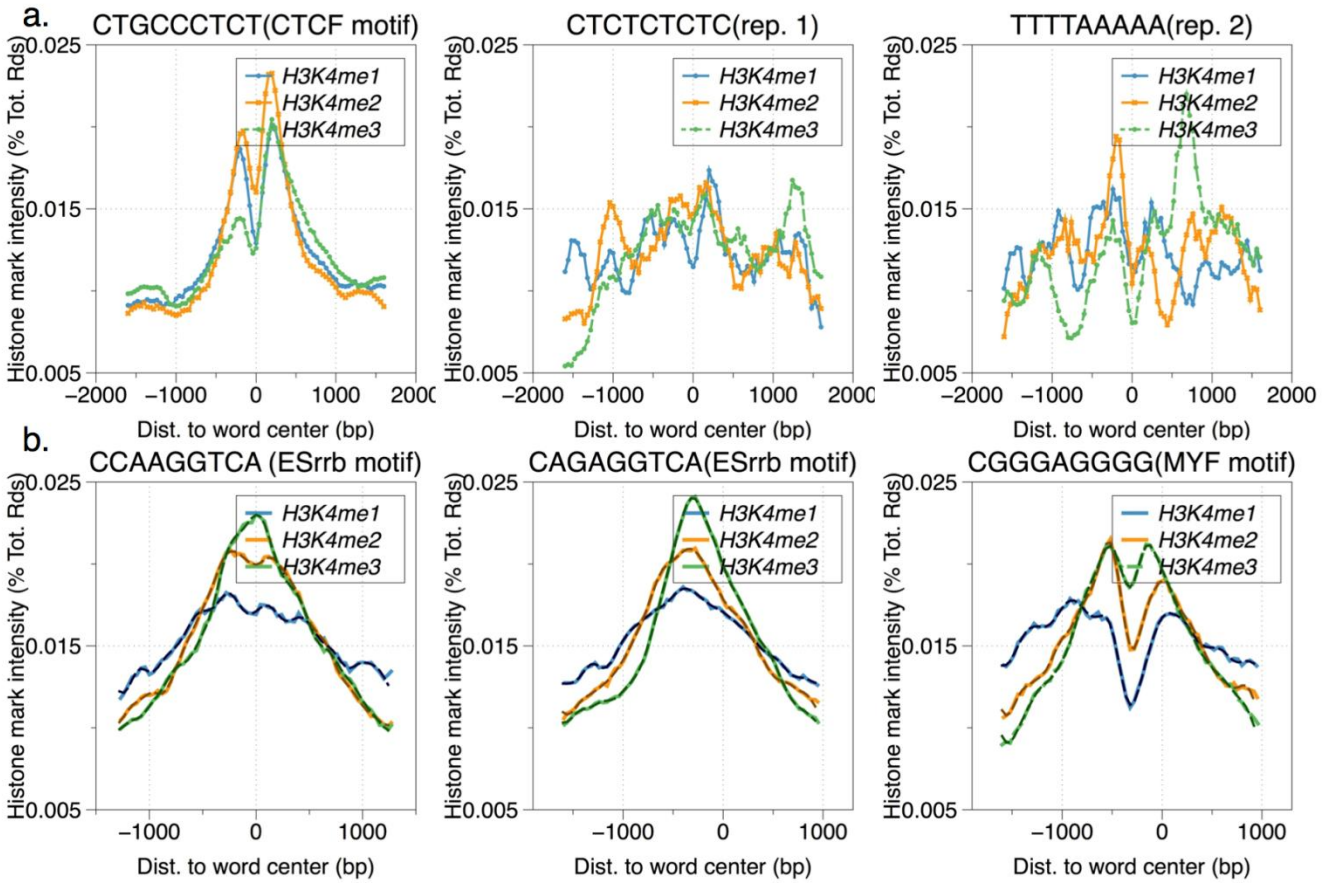
Expectation Maximization (MEME)

- Initial PWM (at random or from average over all potential sites)
- Using current PWM determine probability of all positions being sites
- Re-estimate PWM based on those probabilities
- Continue until convergence – always convergences
- Objective is LLR

Gibbs Sampling

- Similar to EM, but some important differences
- At each iteration pick one site on each seq, chosen by its probability, to update PWM
- Not guaranteed to converge, but tends to increase objective (IC) and plauteau
- Can escape local optima
  - Other MCMC algorithms
    - Metropolis
    - Simulated annealing

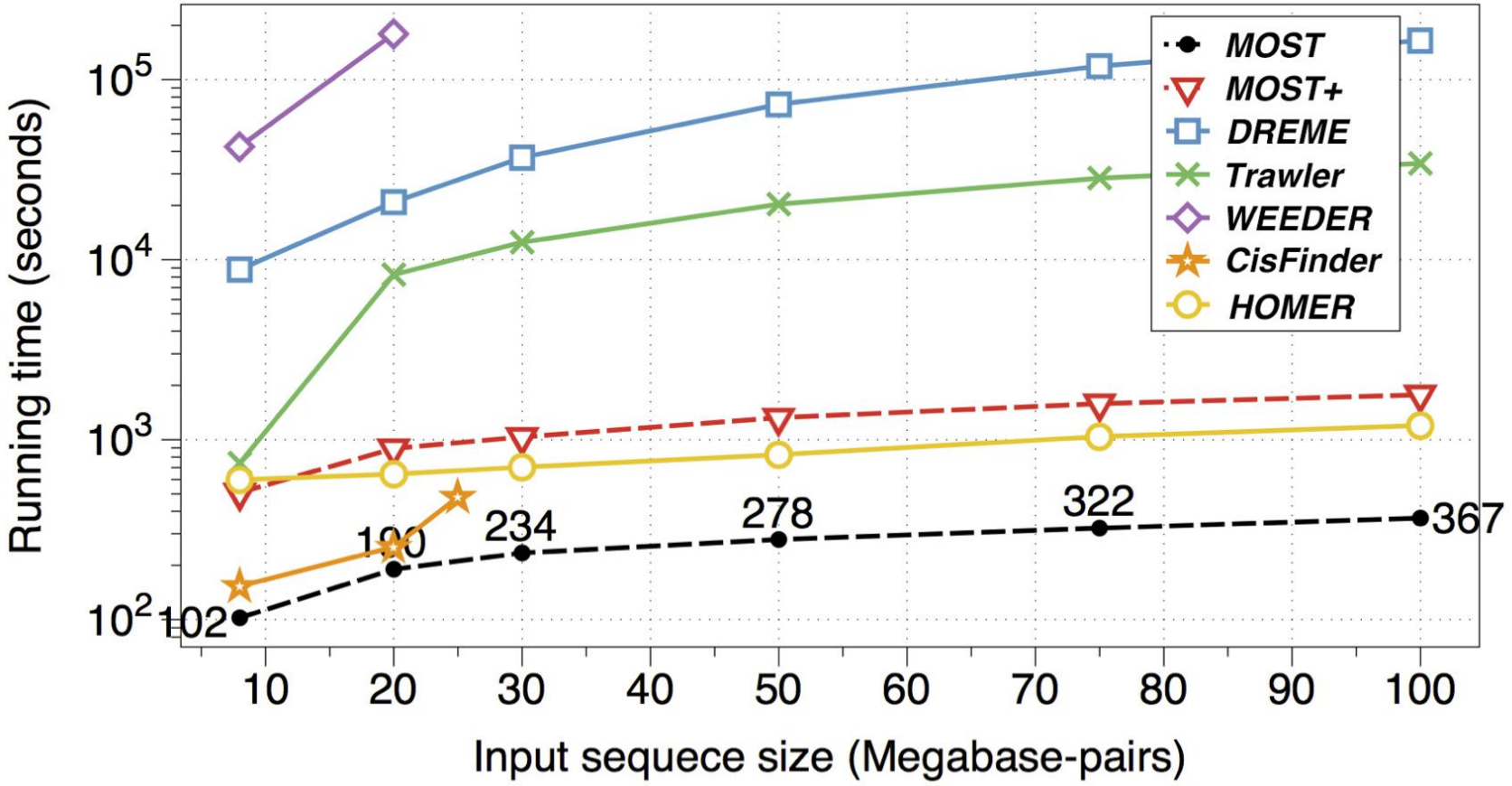
Motif finding by combining genomic sequence and heterogeneous genome-wide signatures



Distributions of several highly enriched word instances found in CTCF and ESrrb's ChIP-seq data set:

Zhang, Y.Z., He, Y.P., Zheng, G.Y., Wei, C.C., BMC Genomics, in press

Motif finding by combining genomic sequence and heterogeneous genome-wide signatures



Comparison of different motif finding methods

Zhang, Y.Z., He, Y.P., Zheng, G.Y., Wei, C.C., BMC Genomics, in press

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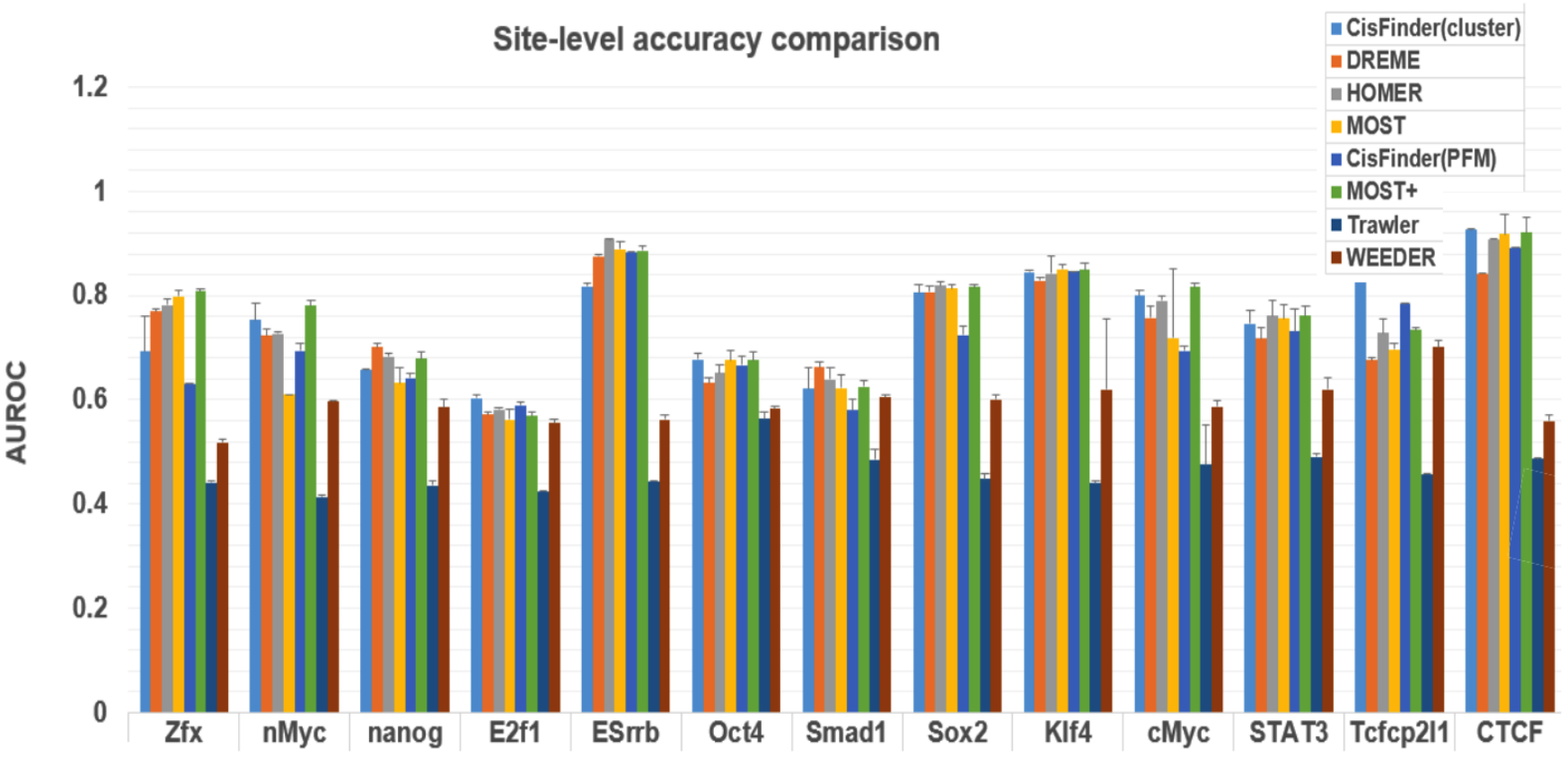

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