

Learning Sequence Motif Models Using Expectation Maximization (EM)

BMI/CS 776

www.biostat.wisc.edu/bmi776/

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Goals for Lecture

Key concepts

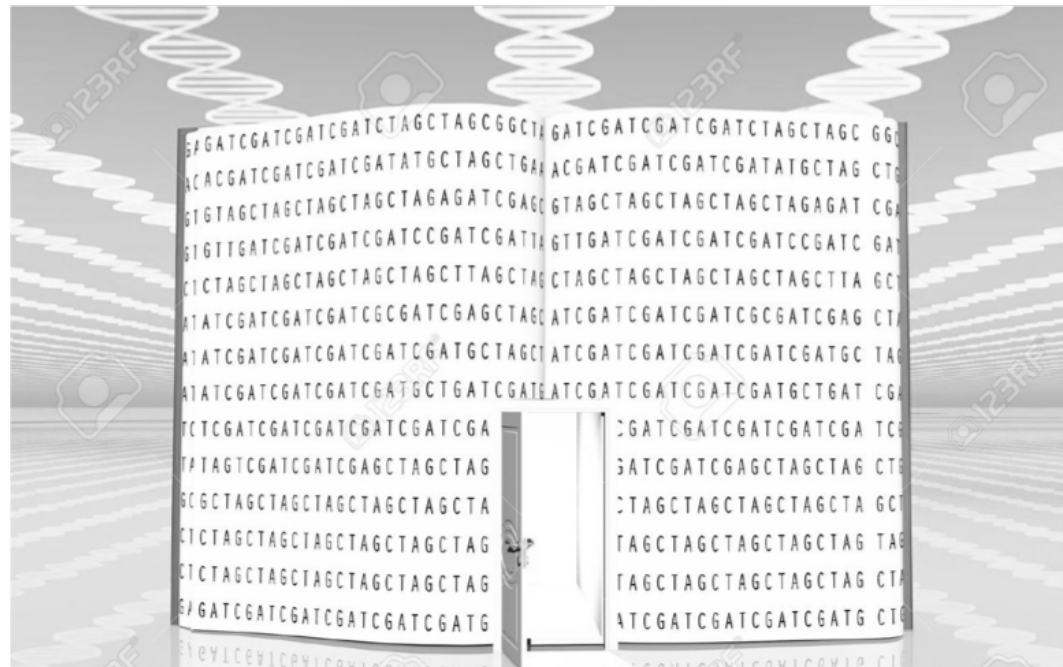
- the motif finding problem
- using EM to address the motif-finding problem
- the OOPS and ZOOPS models

Your genome is your genetic code book

Book	Genome
Chapters	Chromosomes
Sentences	Genes
Words	Elements
Letters	Bases

Human

- 46 chromosomes
- ~ 20,000 – 25,000 genes
- ~ Millions elements
- 4 unique bases (A, T, C, G), ~3 billion in total



<https://goo.gl/images/vMaz4T>

THE #1 NEW YORK TIMES BESTSELLER

BARACK OBAMA

The AUDACITY *of* HOPE

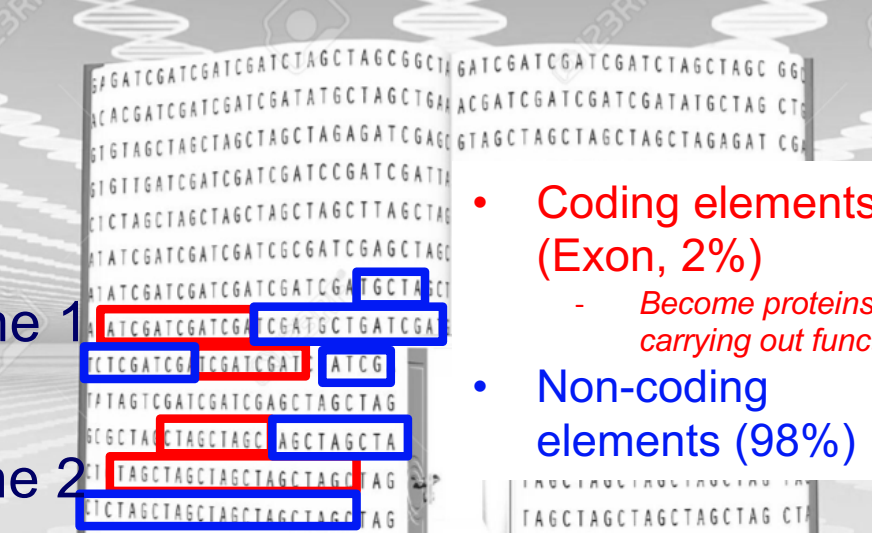
THOUGHTS ON RECLAIMING THE AMERICAN DREAM

From the Author of the #1 New York Times Bestselling
DREAMS FROM MY FATHER



- Key words
- Non-key words

And in gentle steps, one hundred mahogany desks rise from the well of the Senate in four horseshoe-shaped rows. Some of these desks date back to 1819, and atop each desk is a tidy receptacle for inkwells and quills. Open the drawer of any desk, and you will find within the names of the senators who once used it—Taft and Long, Stennis and Kennedy—scratched or penned in the senator's own hand. Sometimes, standing there in



Gene 1

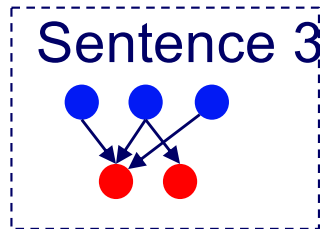
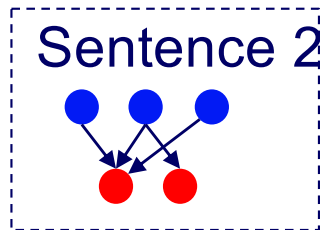
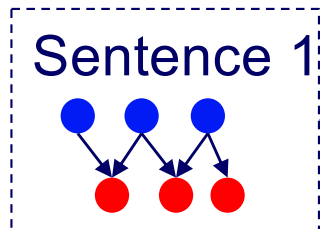
Gene 2

- Coding elements (Exon, 2%)
 - Become proteins carrying out functions
- Non-coding elements (98%)

<https://goo.gl/images/vMaz4T>

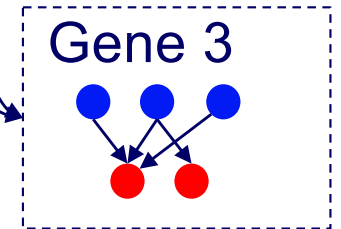
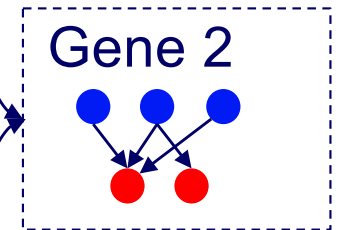
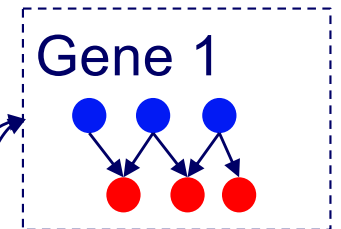
Grammar for book is clear but not for genome

Book	Genome
Chapters	Chromosomes
Sentences	Genes
Words	Elements
Letters	Bases



- Key words
- Non-key words

Grammar → **Functions** ← Pattern



- Coding elements
 - Non-coding elements

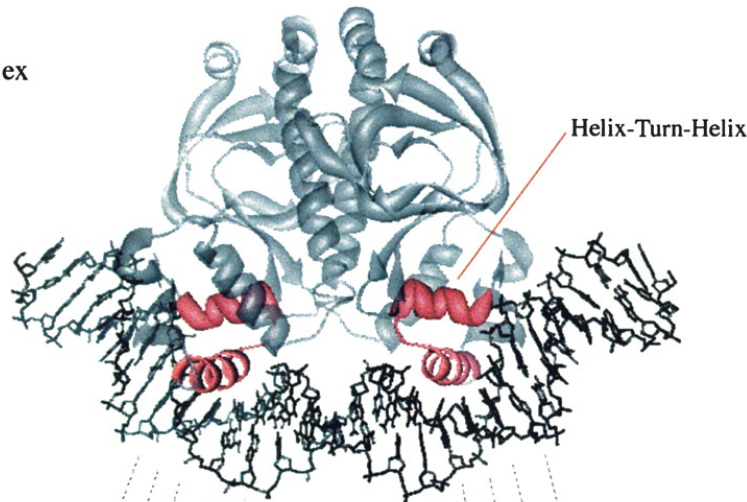
- Set up “rules” in translating genetic codes to functions
- Broken rules -> Abnormal functions
- Unclear

Sequence Motifs

- What is a sequence *motif* ?
 - a sequence pattern of biological significance
- Examples
 - DNA sequences corresponding to protein binding sites
 - protein sequences corresponding to common functions or conserved pieces of structure

Sequence Motifs Example

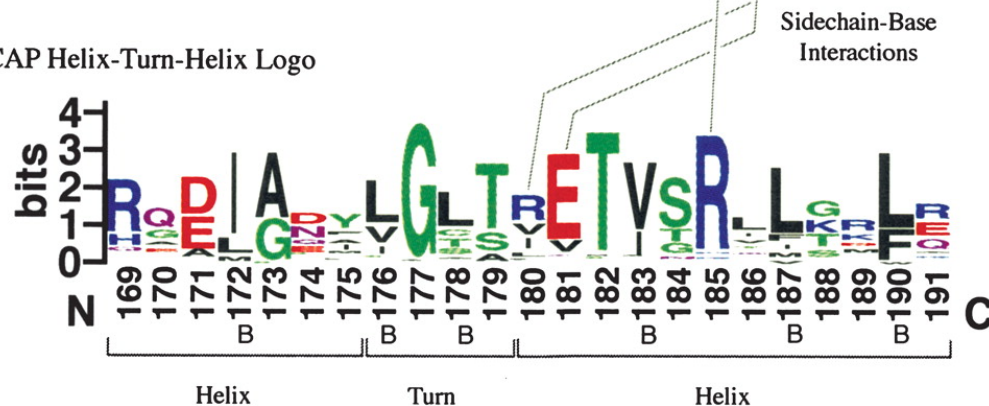
A CAP-DNA Complex



B CAP recognition site DNA Logo



C CAP Helix-Turn-Helix Logo



CAP-binding motif model
based on 59 binding sites in
E.coli

helix-turn-helix motif model
based on 100 aligned protein
sequences

Crooks et al., *Genome Research* 14:1188-90, 2004.

The Motif Model Learning Task

given: a set of sequences that are thought to contain occurrences of an unknown motif of interest

do:

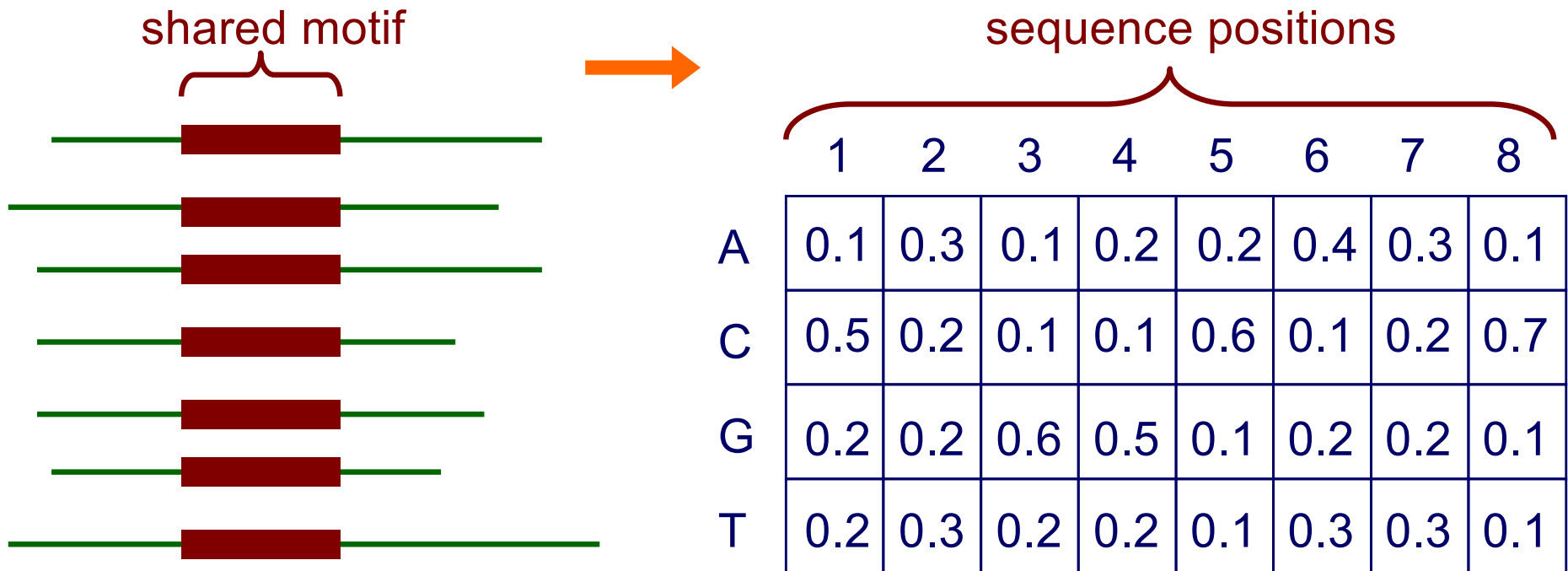
- infer a model of the motif
- predict the locations of the motif occurrences in the given sequences

Why is this important?

- To further our understanding of which regions of sequences are “functional”
- DNA: biochemical mechanisms by which the expression of genes are regulated
- Proteins: which regions of proteins interface with other molecules (e.g., DNA binding sites)
- Mutations in these regions may be significant (e.g., non-coding variants)

Motifs and *Profile Matrices* (a.k.a. *Position Weight Matrices*)

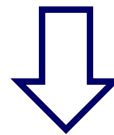
- Given a set of aligned sequences, it is straightforward to construct a profile matrix characterizing a motif of interest



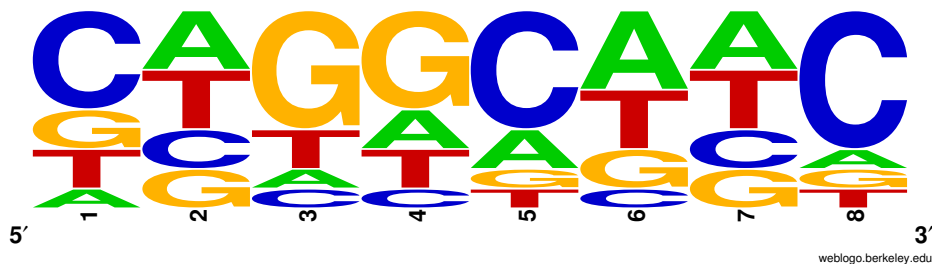
- Each element represents the probability of given character at a specified position

Sequence Logos

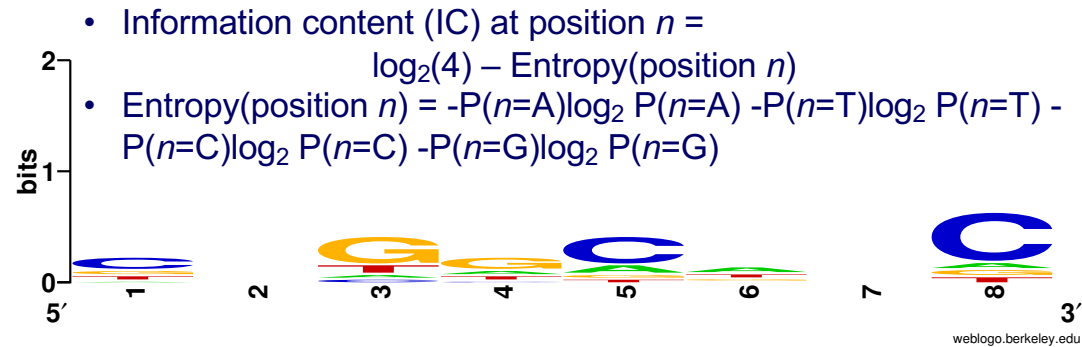
	1	2	3	4	5	6	7	8
A	0.1	0.3	0.1	0.2	0.2	0.4	0.3	0.1
C	0.5	0.2	0.1	0.1	0.6	0.1	0.2	0.7
G	0.2	0.2	0.6	0.5	0.1	0.2	0.2	0.1
T	0.2	0.3	0.2	0.2	0.1	0.3	0.3	0.1



or



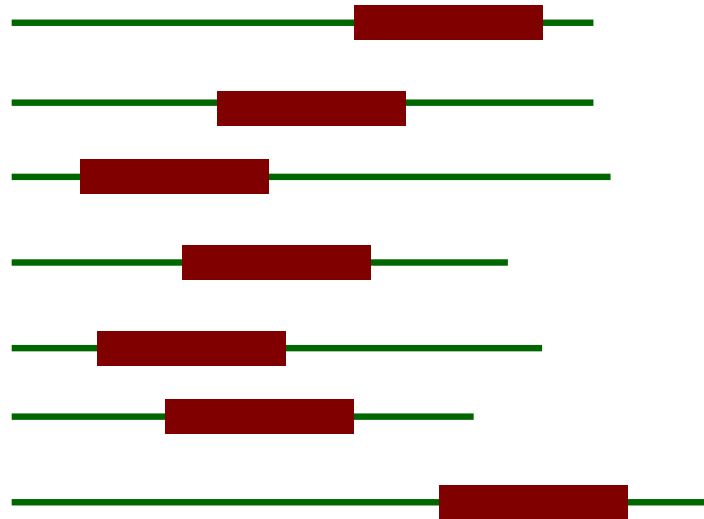
frequency logo



information content logo

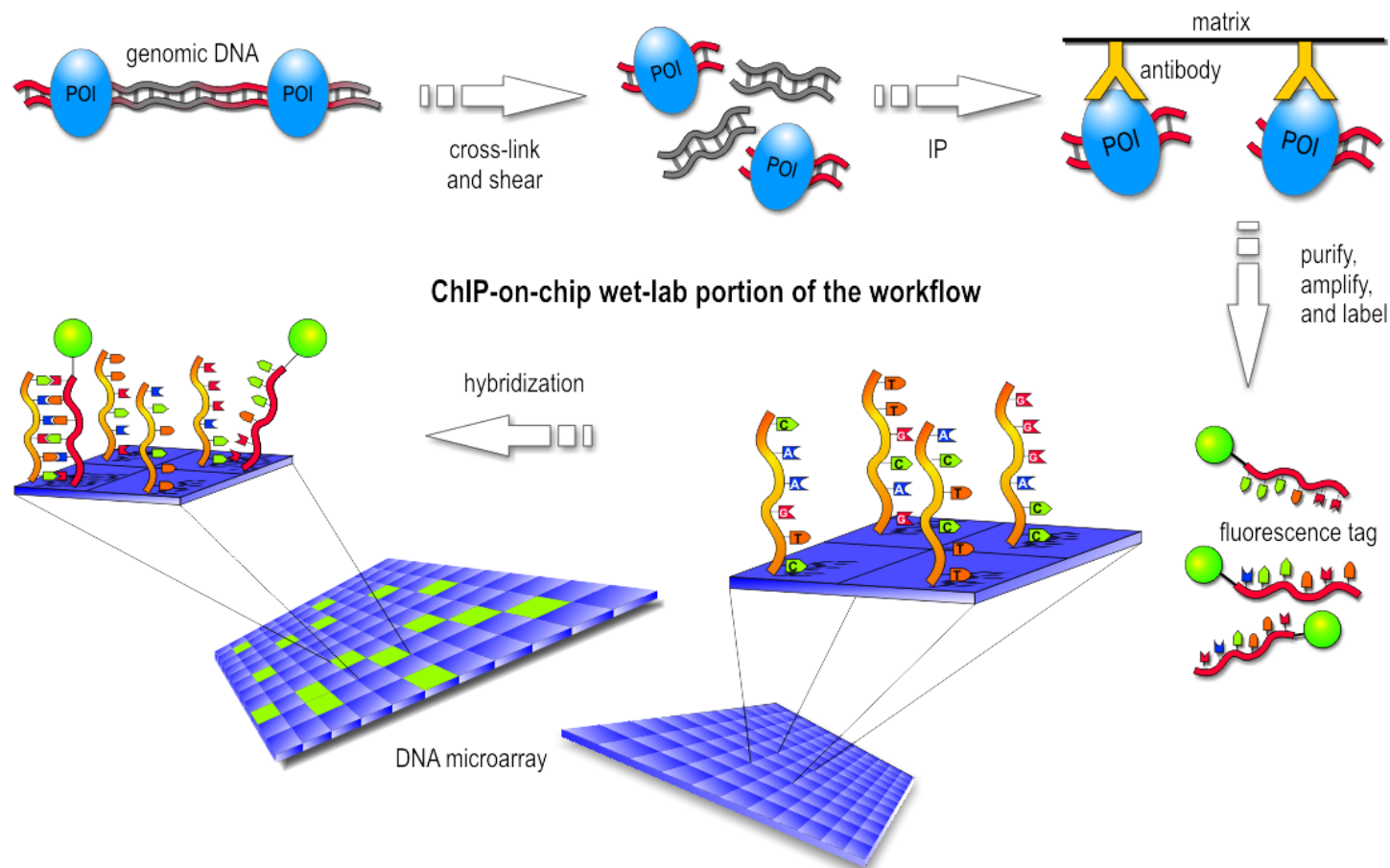
Motifs and Profile Matrices

- How can we construct the profile if the sequences aren't aligned?
- In the typical case we don't know what the motif looks like.



Unaligned Sequence Example

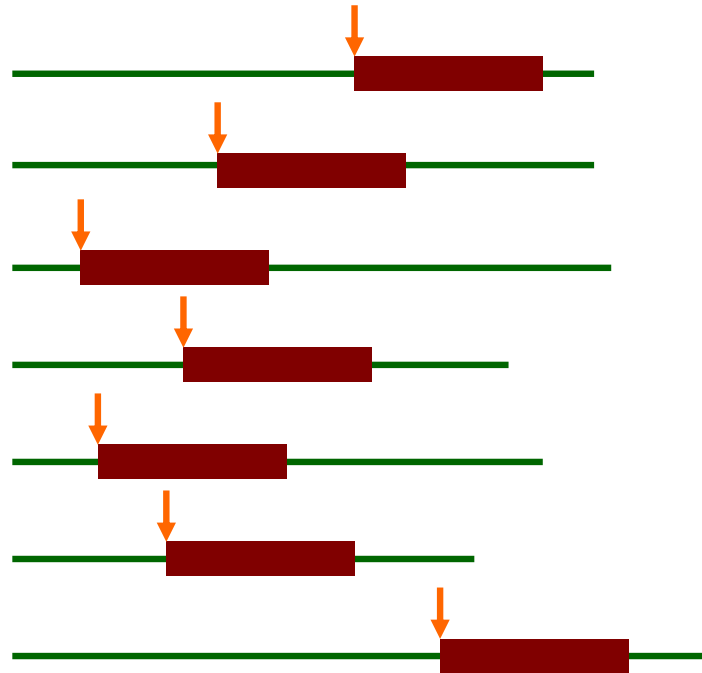
- ChIP-chip experiment tells which probes are bound (though this protocol has been replaced by ChIP-seq)



The Expectation-Maximization (EM) Approach

[Lawrence & Reilly, 1990; Bailey & Elkan, 1993, 1994, 1995]

- EM is a family of algorithms for learning probabilistic models in problems that involve *hidden state*
- In our problem, the hidden state is where the motif starts in each training sequence



Overview of EM

- Method for finding the maximum likelihood (ML) parameters (θ) for a model (M) and data (D)

$$\theta_{ML} = \operatorname{argmax}_{\theta} P(D \mid \theta, M)$$

- Useful when
 - it is **difficult** to optimize $P(D \mid \theta)$ **directly**
 - likelihood can be decomposed by the introduction of **hidden information** (Z)

$$P(D \mid \theta) = \sum_Z P(D, Z \mid \theta)$$

- and it is **easy** to optimize the function (with respect to θ):

$$Q(\theta \mid \theta^t) = \sum_Z P(Z \mid D, \theta^t) \log P(D, Z \mid \theta)$$

(see optional reading and text section 11.6 for details)

Proof of EM algorithm

$$P(D, z|\theta) = P(z|D, \theta)P(D|\theta) \\ \Rightarrow \log P(D|\theta) = \log P(D, z|\theta) - \log P(z|D, \theta)$$

Multiple $P(z|D, \theta^t)$ for given θ^t at both sides and sum over all z values

$$\Rightarrow \log P(D|\theta) \\ = \underbrace{\sum_z P(z|D, \theta^t) \log P(D, z|\theta)}_{Q(\theta|\theta^t)} - \sum_z P(z|D, \theta^t) \log P(z|D, \theta)$$

$$\Rightarrow \log P(D|\theta) - \log P(D|\theta^t) \\ = Q(\theta|\theta^t) - Q(\theta^t|\theta^t) + \underbrace{\sum_z P(z|D, \theta^t) \log \frac{P(z|D, \theta^t)}{P(z|D, \theta)}}_{\text{Non-negative}}$$

$$\Rightarrow \boxed{\log P(D|\theta) - \log P(D|\theta^t) \geq Q(\theta|\theta^t) - Q(\theta^t|\theta^t)}$$

(see optional reading and text section 11.6 for details)

Applying EM to the Motif Finding Problem

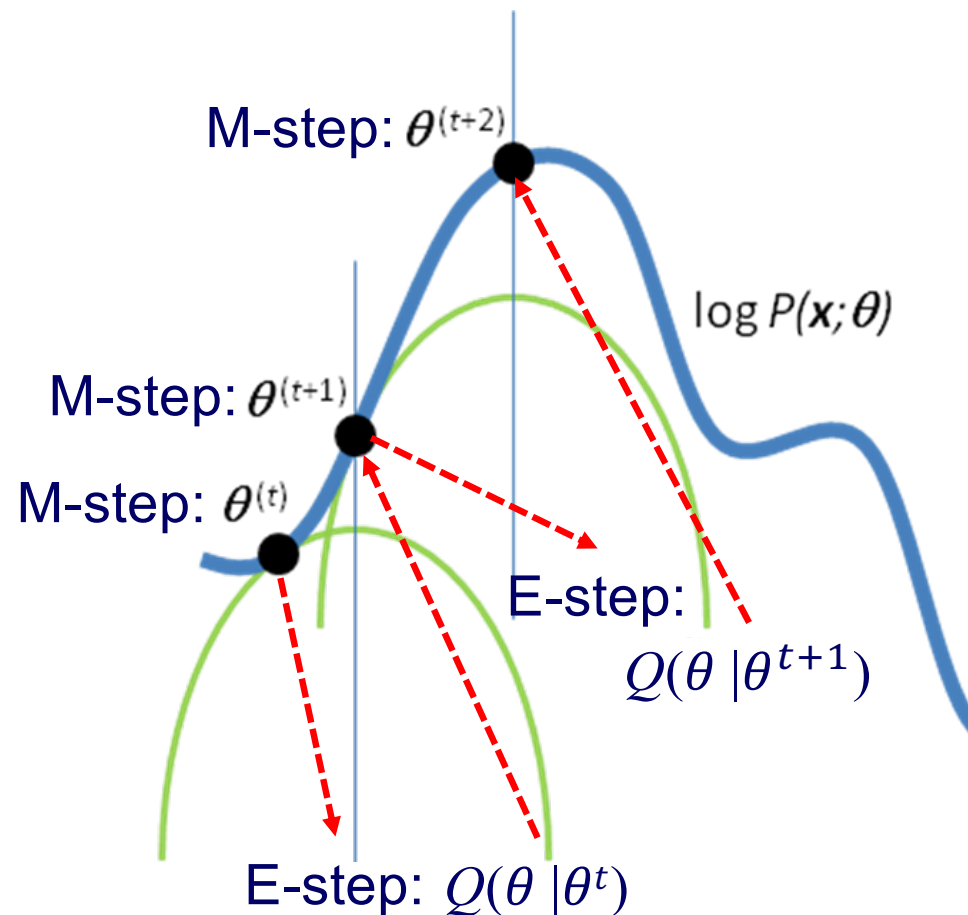
- First define the probabilistic model and likelihood function $P(D | \theta)$
- Identify the hidden variables (Z)
 - In this application, they are the locations of the motifs
- Write out the Expectation (E) step
 - Compute the expected values of the hidden variables given current parameter values θ^t

$$Q(\theta | \theta^t) = \sum_Z P(Z | D, \theta^t) \log P(D, Z | \theta)$$

- Write out the Maximization (M) step
 - Determine the parameters that maximize the Q function, given the expected values of the hidden variables

$$\theta^{t+1} = \operatorname{argmax}_{\theta} Q(\theta | \theta^t)$$

Convergence of the EM algorithm



Representing Motifs in MEME

- MEME: **M**ultiple **EM** for **M**otif **E**licitation
- A motif is
 - assumed to have a fixed width, W
 - represented by a matrix of probabilities: $p_{c,k}$
represents the probability of character c in column k
- Also represent the “background” (i.e. sequence outside the motif): $p_{c,0}$ represents the probability of character c in the background
- Data D is a collection of sequences, denoted X

Representing Motifs in MEME

- Example: a motif model of length 3

$p =$

	0	1	2	3
A	0.25	0.1	0.5	0.2
C	0.25	0.4	0.2	0.1
G	0.25	0.3	0.1	0.6
T	0.25	0.2	0.2	0.1

				
	background		motif positions	

Representing Motif Starting Positions in MEME

- The element $Z_{i,j}$ of the matrix Z is an indicator random variable that takes value 1 if the motif starts in position j in sequence i (and takes value 0 otherwise)
- Example: given DNA sequences where $L=6$ and $W=3$
- Possible starting positions $m = L - W + 1$

G T C A G G

G A G A G T

A C G G A G

C C A G T C

Z =

	1	2	3	4
seq1	0	0	1	0
seq2	1	0	0	0
seq3	0	0	0	1
seq4	0	1	0	0

Probability of a Sequence Given a Motif Starting Position



$$P(X_i \mid Z_{i,j} = 1, p) = \underbrace{\prod_{k=1}^{j-1} p_{c_k, 0}}_{\text{before motif}} \underbrace{\prod_{k=j}^{j+W-1} p_{c_k, k-j+1}}_{\text{motif}} \underbrace{\prod_{k=j+W}^L p_{c_k, 0}}_{\text{after motif}}$$

X_i is the i th sequence

$Z_{i,j}$ is 1 if motif starts at position j in sequence i

c_k is the character at position k in sequence i

Sequence Probability Example

$$X_i = \text{G C } \boxed{\text{T G T}} \text{ A G}$$

$$p = \begin{array}{c} \begin{array}{ccccc} & 0 & 1 & 2 & 3 \\ \text{A} & 0.25 & 0.1 & 0.5 & 0.2 \\ \text{C} & 0.25 & 0.4 & 0.2 & 0.1 \\ \text{G} & 0.25 & 0.3 & 0.1 & 0.6 \\ \text{T} & 0.25 & 0.2 & 0.2 & 0.1 \end{array} \end{array}$$

$$P(X_i \mid Z_{i,3} = 1, p) =$$

$$\begin{aligned} & p_{\text{G},0} \times p_{\text{C},0} \times p_{\text{T},1} \times p_{\text{G},2} \times p_{\text{T},3} \times p_{\text{A},0} \times p_{\text{G},0} = \\ & 0.25 \times 0.25 \times 0.2 \times 0.1 \times 0.1 \times 0.25 \times 0.25 \end{aligned}$$

Likelihood Function

- EM (indirectly) optimizes log likelihood of observed data

$$\log P(X | p)$$

- M step requires joint log likelihood

$$\begin{aligned}\log P(X, Z | p) &= \log \prod_i P(X_i, Z_i | p) \\ &= \log \prod_i P(X_i | Z_i, p) P(Z_i | p) \\ &= \log \prod_i \frac{1}{m} \prod_j P(X_i | Z_{i,j} = 1, p)^{Z_{i,j}} \\ &= \sum_i \sum_j Z_{i,j} \log P(X_i | Z_{i,j} = 1, p) + n \log \frac{1}{m}\end{aligned}$$

See Section IV.C of [Bailey's dissertation](#) for details

Basic EM Approach

given: length parameter W , training set of sequences

$t=0$

set initial values for $p^{(0)}$

do

$++t$

 re-estimate $Z^{(t)}$ from $p^{(t-1)}$ (E-step)

 re-estimate $p^{(t)}$ from $Z^{(t)}$ (M-step)

 until change in $p^{(t)} < \varepsilon$ (or change in likelihood is $< \varepsilon$)

return: $p^{(t)}, Z^{(t)}$

Expected Starting Positions

- During the E-step, we compute the expected values of Z given X and $p^{(t-1)}$
- We denote these expected values $Z^{(t)} = E[Z \mid X, p^{(t-1)}]$
- For example:

indicator random variable

expected value at iteration t

		1	2	3	4
$Z^{(t)} =$	seq1	0.1	0.1	0.2	0.6
	seq2	0.4	0.2	0.1	0.3
	seq3	0.3	0.1	0.5	0.1

The E-step: Computing $Z^{(t)}$

- To estimate the starting positions in Z at step t

$$Z_{i,j}^{(t)} = \frac{P(X_i | Z_{i,j} = 1, p^{(t-1)})P(Z_{i,j} = 1)}{\sum_{k=1}^m P(X_i | Z_{i,k} = 1, p^{(t-1)})P(Z_{i,k} = 1)}$$

- This comes from Bayes' rule applied to

$$P(Z_{i,j} = 1 | X_i, p^{(t-1)})$$

The E-step: Computing $Z^{(t)}$

- Assume that it is equally likely that the motif will start in any position

$$P(Z_{i,j} = 1) = \frac{1}{m}$$

$$Z_{i,j}^{(t)} = \frac{P(X_i | Z_{i,j} = 1, p^{(t-1)}) \cancel{P(Z_{i,j} = 1)}}{\sum_{k=1}^m P(X_i | Z_{i,k} = 1, p^{(t-1)}) \cancel{P(Z_{i,k} = 1)}}$$

Example: Computing $Z^{(t)}$

$$X_i = \text{G C T G T A G}$$

$$p^{(t-1)} = \begin{array}{c} \begin{array}{cc} & \begin{array}{cccc} & 0 & 1 & 2 & 3 \end{array} \\ \begin{array}{c} \text{A} \\ \text{C} \\ \text{G} \\ \text{T} \end{array} & \begin{array}{cccc} 0.25 & 0.1 & 0.5 & 0.2 \\ 0.25 & 0.4 & 0.2 & 0.1 \\ 0.25 & 0.3 & 0.1 & 0.6 \\ 0.25 & 0.2 & 0.2 & 0.1 \end{array} \end{array} \end{array}$$

$$Z^{(t)}_{i,1} \propto P(X_i | Z_{i,1} = 1, p^{(t-1)}) = 0.3 \times 0.2 \times 0.1 \times 0.25 \times 0.25 \times 0.25 \times 0.25$$

$$Z^{(t)}_{i,2} \propto P(X_i | Z_{i,2} = 1, p^{(t-1)}) = 0.25 \times 0.4 \times 0.2 \times 0.6 \times 0.25 \times 0.25 \times 0.25$$

⋮

- Then normalize so that $\sum_{j=1}^m Z^{(t)}_{i,j} = 1$

The M-step: Estimating p

- Recall $p_{c,k}$ represents the probability of character c in position k ; values for $k=0$ represent the background

$$p_{c,k}^{(t)} = \frac{n_{c,k} + d_{c,k}}{\sum_{b \in \{A,C,G,T\}} (n_{b,k} + d_{b,k})}$$

pseudo-counts

$$n_{c,k} = \begin{cases} \sum_i \sum_{\{j | X_{i,j+k-1}=c\}} Z_{i,j}^{(t)} & k > 0 \\ n_c - \sum_{j=1}^W n_{c,j} & k = 0 \end{cases}$$

of c 's at position k → $n_{c,k}$

total # of c 's in data set → n_c

sum over positions where c appears

Example: Estimating p

A C A G C A

$$Z^{(t)}_{1,1} = 0.1, Z^{(t)}_{1,2} = 0.7, Z^{(t)}_{1,3} = 0.1, Z^{(t)}_{1,4} = 0.1$$

A G G C A G

$$Z^{(t)}_{2,1} = 0.4, Z^{(t)}_{2,2} = 0.1, Z^{(t)}_{2,3} = 0.1, Z^{(t)}_{2,4} = 0.4$$

T C A G T C

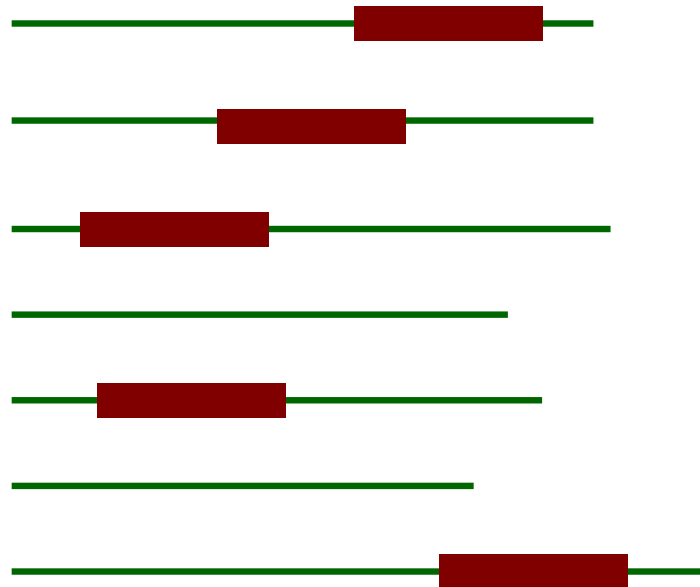
$$Z^{(t)}_{3,1} = 0.2, Z^{(t)}_{3,2} = 0.6, Z^{(t)}_{3,3} = 0.1, Z^{(t)}_{3,4} = 0.1$$

$$p^{(t)}_{A,1} = \frac{Z^{(t)}_{1,1} + Z^{(t)}_{1,3} + Z^{(t)}_{2,1} + Z^{(t)}_{3,3} + 1}{Z^{(t)}_{1,1} + Z^{(t)}_{1,2} \dots + Z^{(t)}_{3,3} + Z^{(t)}_{3,4} + 4}$$

$$p^{(t)}_{C,2} = \frac{Z^{(t)}_{1,1} + Z^{(t)}_{1,4} + Z^{(t)}_{2,3} + Z^{(t)}_{3,1} + 1}{Z^{(t)}_{1,1} + Z^{(t)}_{1,2} \dots + Z^{(t)}_{3,3} + Z^{(t)}_{3,4} + 4}$$

The ZOOPS Model

- The approach as we've outlined it, assumes that each sequence has exactly one motif occurrence per sequence; this is the OOPS model
- The ZOOPS model assumes zero or one occurrences per sequence



E-step in the ZOOPS Model

- We need to consider another alternative: the i th sequence doesn't contain the motif
- We add another parameter (and its relative)

γ

- prior probability of a sequence containing a motif

$$\lambda = \frac{\gamma}{(L - W + 1)} = \frac{\gamma}{m}$$

- prior probability that any position in a sequence is the start of a motif

- Possible starting positions $m = L - W + 1$

E-step in the ZOOPS Model

$$Z_{i,j}^{(t)} = \frac{P(X_i | Z_{i,j} = 1, p^{(t-1)})\lambda^{(t-1)}}{P(X_i | Q_i = 0, p^{(t-1)})(1 - \gamma^{(t-1)}) + \sum_{k=1}^m P(X_i | Z_{i,k} = 1, p^{(t-1)})\lambda^{(t-1)}}$$

- Q_i is a random variable for which $Q_i = 1$ if sequence X_i contains a motif, $Q_i = 0$ otherwise

$$Q_i = \sum_{j=1}^m Z_{i,j}$$

$$P(X_i | Q_i = 0, p^{(t-1)}) = \prod_{j=1}^L p_{c_j,0}^{(t-1)} \quad P(Q_i = 0) = 1 - \gamma^{(t-1)}$$

M-step in the ZOOPS Model

- Update p same as before
- Update γ as follows:

$$\gamma^{(t)} \equiv m\lambda^{(t)} = \frac{1}{n} \sum_{i=1}^n Q_i^{(t)}$$

Extensions to the Basic EM Approach in MEME

- Varying the approach (TCM model) to assume *zero or more* motif occurrences per sequence
- Choosing the width of the motif
- Finding multiple motifs in a group of sequences
- ✓ Choosing good starting points for the parameters
- ✓ Using background knowledge to bias the parameters

Starting Points in MEME

- EM is susceptible to local maxima, so it's a good idea to try multiple starting points
- Insight: motif must be similar to *some* subsequence in data set
- For every distinct subsequence of length W in the training set
 - derive an initial p matrix from this subsequence
 - run EM for 1 iteration
- Choose motif model (i.e. p matrix) with highest likelihood
- Run EM to convergence

Using Subsequences as Starting Points for EM

- Set values matching letters in the subsequence to some value π
- Set other values to $(1 - \pi)/(M-1)$ where M is the length of the alphabet
- Example: for the subsequence TAT with $\pi = 0.7$

$$p = \begin{array}{c} \begin{array}{ccccc} & & 1 & 2 & 3 \\ \mathbf{A} & 0.1 & 0.7 & 0.1 \\ \mathbf{C} & 0.1 & 0.1 & 0.1 \\ \mathbf{G} & 0.1 & 0.1 & 0.1 \\ \mathbf{T} & 0.7 & 0.1 & 0.7 \end{array} \end{array}$$

MEME web server

MEME Suite 4.11.0

- Motif Discovery
- Motif Enrichment
- Motif Scanning
- Motif Comparison
- Manual
- Guides & Tutorials
- Sample Outputs
- File Format Reference
- Databases
- Download & Install
- Help
- Alternate Servers
- ▼ Authors & Citing
 - Authors
 - Citing the MEME Suite
- Recent Jobs
- ↔ Previous version 4.10.2

**MEME**
Multiple Em for Motif Elicitation
Version 4.11.0

MEME discovers novel, **ungapped** motifs (recurring, fixed-length patterns) in your sequences (sample output from sequences). MEME splits variable-length patterns into two or more separate motifs. See this Manual for more information.

Data Submission Form

Perform motif discovery on DNA, RNA or protein datasets.

Select the motif discovery mode
☒ Normal mode ☐ Discriminative mode [?](#)

Select the sequence alphabet
Use sequences with a standard alphabet or specify a custom alphabet. [?](#)
☒ DNA, RNA or Protein ☐ Custom

Input the primary sequences
Enter sequences in which you want to find motifs. [?](#)
 [?](#)

Select the site distribution
How do you expect motif sites to be distributed in sequences? [?](#)

Select the number of motifs
How many motifs should MEME find? [?](#)

Input job details
(Optional) Enter your email address. [?](#)

(Optional) Enter a job description. [?](#)

► **Advanced options**

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

Version 4.11.0Please send comments and questions to: meme-suite@uw.eduPowered by Opal

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<http://meme-suite.org/>