

Introduction to Bioinformatics

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Local Multiple Sequence Alignment Sequence File Formats

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Localized Alignments

- Just like with pairwise alignments, we may not be interested in the global alignment of multiple sequences, but rather only specific regions that are conserved.
- Local Alignment of **MSAs** are important:
 - Given regions of genomic DNA occurring upstream or before a certain gene, there might be sequences where transcription factors bind to the DNA so that the gene can be transcribed.
 - Thus, if we are interested in determining if there is any signal in the regions upstream of a certain family of genes across several different organisms, it would be important to only find the conserved region, and not try to align all of the genomic DNA.
 - Localized alignments of protein sequences can yield information about conserved domains found in otherwise unrelated proteins.

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Approaches to Local Alignment

- Profile Analysis
- Block Analysis
- Pattern-searching or statistical methods

Profile Analysis...

- Profiles are found by first multiply aligning the sequences, determining which regions are the most highly conserved,
- and then creating a scoring matrix for the alignment of the highly conserved region.
- Profile is composed of:
 - **Columns:**
 - one for each residue;
 - columns for insertions and deletions as well
 - **Rows:**
 - one for each position in the conserved region or motif

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...Profile Analysis

- Profiles describe a MSA by a scoring matrix:

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```

Drawback to Profiles

- Profiles only as representative as the variation in the training sets.
- Thus, there is a bias in the profile towards the training data.
- Training sets can be erroneous if not carefully constructed

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Calculating Profiles

- Each cell is the **log-odds** score
 - The value of an individual cell is calculated as the log odds score of finding a particular residue in a particular location in an alignment divided by the probability of aligning the two amino acids by random chance using a particular scoring scheme (such as PAM250, BLOSUM80, ...).
 - PAM (Percent Accepted Mutation)
 - BLOSUM (Blocks Substitution Matrix)
 - Additional penalties must be calculated for gap opening and gap extension in the profile as well.
- Some methods take in sequence weights as well
 - One method (average method) weighs the proportion of the amino acids found in a particular column, and weights the score of matching the consensus residue at a given position to that particular residue.

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Shannon Entropy

- One method to calculate the observed column variation given the expected variation in the evolutionary model is to use an information measure known as **entropy**.
 - Entropy is the amount of information of the observed column variation if expected variation in the evolutionary model is known
- The smaller the entropy, the more conserved a column is.

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Entropy...

- The entropy (**H**) for a single column is calculated by the following formula:

$$H = - \sum_{\text{residues}(a)} f_a \log(p_a)$$

- **a**: is a residue (amino acid),
- **f_a**: frequency of residue **a** in a column,
- **p_a**: probability (expected frequency) of residue **a** in that column

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...Entropy...

- **H** is calculated for each 20 ancestor amino acids and for a large number of evolutionary distances (PAM1, PAM2, PAM4, ...).
- The distance that gives the minimum value for **H** for each column-possible ancestor combination is the best estimate of the distance that generates the column diversity from that ancestor.
- This analysis provides 20 possible models (**M_a** for **a** = 1,2,3,...,20) as to how the amino acid frequencies in a column (**F**) may have originated.

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...Entropy...

- The next step in the evolutionary profile construction determines the extent to which each **M_a** predicts **F** by the Bayes conditional probability analysis.

$$P(M_a|F) = P(M_a) \times P(F|M_a) / \sum_{\text{all } a's} P(M_a) \times P(F|M_a)$$

- where the prior distribution **P(M_a)** is given by the background amino acid frequencies and

$$P(F|M_a) = P_{aa1}^{faa1} \times P_{aa2}^{faa2} \times P_{aa3}^{faa3} \dots P_{aa20}^{faa20}$$

- i.e., the product of the expected amino acid frequencies in **M_a** raised to the power of the fraction observed for each amino acid in the msa column.

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...Entropy

- From $P(M_a|F)$, the weights for each of the 20 possible distributions that give rise to the msa column diversity are calculated as follows:

$$W_a = P(M_a|F) - P(M_{random}|F)$$

- where W_a is the weight given to M_a and $P(M_{random}|F)$ is calculated as above using amino acid distribution.

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Log-odds score...

- Another measure of creating a profile is by using **log-odds score**.
- In this method,
 - the \log_2 of the ratio of observed/background frequencies is calculated for each position.
 - What results is the amount of information available in an alignment given in bits.
- A new sequence can then be searched to see if it possibly contains the motif.
- Profiles can also indicate log-odds score:
 - $\text{Log}_2(\text{observed} \div \text{expected})$
- Result is a bit score

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...Log-odds score

- The **log odds scores** for the profile (**Profile_{ij}**) are given by

$$\text{Profile}_{ij} = \log \left[\sum_{\text{all } a's} (W_{ai} \times P_{aij}) / P_{\text{random}j} \right]$$

where

- W_{ai} is the weight of an ancestral amino acid a at row i in the profile,
- P_{aij} is the frequency of amino acid j in the PAM amino acid distribution that best matches at row i ,
- $P_{\text{random}j}$ is the background frequency of amino acid j .

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BLOCK Analysis...

- Blocks are similar to profiles in the sense that
 - they represent locally conserved regions within a MSA.
- However, the difference is that ...
 - blocks lack insert and delete (**indels**) positions in the sequences.
 - Instead, every column includes only matches and mismatches
- Blocks can be determined either
 - by performing a multiple sequence alignment, or
 - by searching a database for similar sequences of the same length.

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...BLOCK Analysis...

- Generally determined by performing multiple alignment first
- Ungapped regions are then separated into blocks
- Algorithms have been developed for searching for blocks

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...BLOCK Analysis

- Statistical approaches to finding the most alike sequences have been proposed, such as
 - the Expectation-Maximization algorithms and
 - the Gibbs sampler.
- In any case, once a set of blocks has been determined, the information contained within the block alignment can be displayed as a sequence profile.

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BLOCKS Programs

- A global sequence alignment will usually contain ungapped regions that are aligned between multiple sequences.
- These regions can be extracted to produce blocks.
- Two widely used programs:
 - BLOCKS**
 - eMOTIF**

http://www.blocks.fhcrc.org/blocks/process_blocks.html

<http://dna.stanford.edu/emotif/>

Example...

- 10 Truncated Kinase proteins
 - Approximately 75 residues in length
 - A protein kinase is a kinase enzyme that modifies other proteins by chemically adding phosphate groups to them (phosphorylation).
 - The human genome contains about 500 protein kinase genes and they constitute about 2% of all human genes.
 - Protein kinases are also found in bacteria and plants.
 - Up to 30% of all human proteins may be modified by kinase activity, and kinases are known to regulate the majority of cellular pathways, especially those involved in signal transduction.

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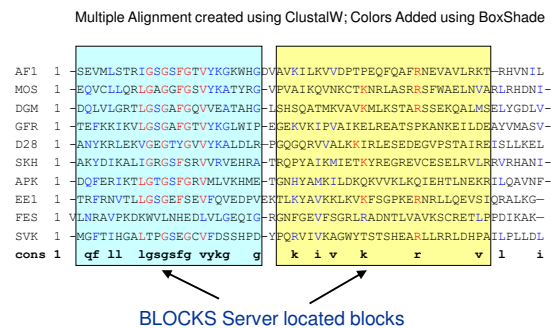
20

...Example...

```
>D28      CD28  S. CEREVISIAE CELL CYCLE CONTROL PROTEIN KINASE
ANYKRLKVGEGTYGVVYKALDLRPFQGGVVAUKKIRLESEDEGVFPSTAIRISLLKEL
>SKH      SKH  HELA MYSTERY PUTATIVE PROTEIN KINASE
AKYDIKALIGRGSFVRVVEHRAITRQPYAIKMIETKYREGREVCESELRLVLRVRHANI
>APK      CAPK BOVINE CARDIAC MUSCLE CYCLIC AMP-DEPENDENT (ALPHA)
DQFERIKTLGTGSFGRVMLVKHMETGNHYAMKILDKQVVKLKQIEHTLNEKRILQAVNF
>EE1      WEE1  S. POMBE MITOTIC INHIBITOR
TRFRNVTLGSGEFSEVFQVEDPVKTLKYAVKKLVKVFSGPKERNRLQEVSIQRLKG
>GFR      EGFR HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR
TEFKKIKVLGSGAFGTIVYKGLWIPGEKVKIPVAIKELREATSPKANKEILDEAYVMASV
>DGM      PDGF RECEPTOR, MOUSE KINASE REGION
DQLVLGRTLGSAGFQGVVEATAHGLSHQATMKVAVMLKSTARSSEKQALMSLYGDLV
>FES      THIS IS VFES TYROSINE KINASE
VLNRAVPKDKWVLNHDVLVGEQIGRNFGEVFSGRRLADNTLVAVKSCRETLPDIKAK
>AF1      RAF1 HUMAN C-RAF-1 ONCOGENE
SEVMLSTRIGSGSFGTVYKKGWHDVAVKI LKVVDPTEPQFQAFRNEAVLRKTRHVNIL
>MOS      CMOS HUMAN C-MOS ONCOGENE
EQVCLLQRLGAGGFGSVYKATYRGVPAIKQVKNCTKNRLASRRSFWAELNVARLRHNDI
>SVK      HSVK HERPES SIMPLEX VIRUS PUTATIVE PROTEIN KINASE
MGFTIHGALTPGSEGCVFDSHPDYPQVRVIVKAGWYTTSTSHEARLLRLDHPAILPLLDL
```

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...Example...



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...Example...

```
* Taking this alignment, blocks can be generated using
the BLOCKS server:

ID      x6676xbli; BLOCK
AC      x6676xbliB; distance from previous blocks=(1,1)
DE      ../tmp/6676.blin
BL      UNK motif; width=24; seqs=10; 99.5%=0; strength=0
AF1      ( 1) SEVMLSTRIGSGSFGTVYKKGWHDVAVKI 41
MOS      ( 1) EQVCLLQRLGAGGFGSVYKATYRG 48
DGM      ( 1) DQLVLGRTLGSAGFQGVVEATAHG 49
GFR      ( 1) TEFKKIKVLGSGAFGTIVYKGLWIP 41
D28      ( 1) ANYKRLKVGEGTYGVVYKALDLR 61
SKH      ( 1) AKYDIKALIGRGSFVRVVEHRA 54
APK      ( 1) DQFERIKTLGTGSFGRVMLVKHME 46
EE1      ( 1) TRFRNVTLGSGEFSEVFQVEDPV 55
FES      ( 1) VLNRAVPKDKWVLNHDVLVGEQIG 100
SVK      ( 1) MGFTIHGALTPGSEGCVFDSHPD 73
//
```

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...Example

```
ID      x6676xbli; BLOCK
AC      x6676xbliB; distance from previous blocks=(2,2)
DE      ../tmp/6676.blin
BL      UNK motif; width=28; seqs=10; 99.5%=0; strength=0
AF1      ( 27) AVKILKVVDPTEPQFQAFRNEAVLRKT 87
MOS      ( 27) PVAIKQVKNCTKNRLASRRSFWAELNVA 75
DGM      ( 27) SHSQATMKVAVMLKSTARSSEKQALMS 92
GFR      ( 27) GEKVKIPVAIKELREATSPKANKEILDE 83
D28      ( 27) PSQQQRVVALKKIRLESEDEGVFPSTAIR 83
SKH      ( 27) TRQPYAIKMIETKYREGREVCESELRLVLR 74
APK      ( 27) TGNHYAMKILDKQVVKLKQIEHTLNEKR 85
EE1      ( 27) TLKYAVKKLVKVFSGPKERNRLQEVSI 77
FES      ( 27) GNFGEVFSGRRLADNTLVAVKSCRETLP 100
SVK      ( 27) PQRVIVKAGWYTTSTSHEARLLRLDHPA 92
//
```

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...Example of EM - Residue Frequencies...

- After calculating the observed counts for each of the positions, we can convert these to observed frequencies:

Nucleotide	Motif Position (0 = Background)						
	0	1	2	3	4	5	6
A	0.267	0.209	0.414	0.209	0.209	0.379	0.241
C	0.267	0.276	0.103	0.172	0.241	0.241	0.241
G	0.216	0.310	0.276	0.345	0.241	0.172	0.276
T	0.250	0.209	0.209	0.276	0.310	0.209	0.241

– Frequency of nucleotide *a* for the background (Col0):

of nucleotide *a* in Col0 in Row*a* / # of all nucleotides in Col0

– Frequency of *a* in Col*c*:

of nucleotide *a* in Col*c* / # of all nucleotides in Col*c*

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...Example of EM - Residue Frequencies...

- However, in order to alleviate the issue of **zero counts** and overtraining of the data, **pseudocounts** are introduced to the observed counts:

– In this case, frequency of nucleotide *a* in Col*c*:

$$P_{ca} = (n_{ca} + b_{ca}) / (N_c + B_c)$$

P_{ca} : Probability of residue *a* in column *c*; n_{ca} : count of *a*'s in column *c*; b_{ca} : pseudocount of *a*'s in column *c*;

N_c : total count in column *c*; B_c : total pseudocount in column *c*

– Choosing a pseudocount is arbitrary

– For example, assuming that 4 nucleotides have equal probabilities, if total pseudocount (B_c) is chosen as 1, pseudocount of each nucleotide will be $b_{ca} = B_c/4$.

– Note that a different pseudocount scheme is used in the following table

Nucleotide	Motif Position (0 = Background)						
	0	1	2	3	4	5	6
A	0.267	0.256	0.296	0.256	0.256	0.289	0.263
C	0.267	0.263	0.230	0.243	0.256	0.256	0.256
G	0.216	0.240	0.233	0.246	0.226	0.213	0.233
T	0.250	0.241	0.241	0.254	0.261	0.241	0.248

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...Example of EM - Maximization Step...

- In the expectation step, the residue frequencies for the motif are used to estimate the composition of the motif site.
- The expectation step attempts to maximally discriminate between sequence within and not within the site.
- For each sequence, each possible motif location is considered in order to find the most probable location given the current motif.
- Consider the first sequence:
 - TCAGAACCGAGTTATATATTCCTTCTCCACTCCT
 - There are 41 residues; 41 - 6 + 1 = 36 sites to consider
- Starting from the first site (TCAGAA), 36 scores for the first sequence are calculated

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...Example of EM - Residue Frequencies...

- Let us consider the eighth site CAGTTA.

– In order to calculate site score, observed frequency table is used:

Nucleotide	Motif Position (0 = Background)						
	0	1	2	3	4	5	6
A	0.267	0.256	0.296	0.256	0.256	0.289	0.263
C	0.267	0.263	0.230	0.243	0.256	0.256	0.256
G	0.216	0.240	0.233	0.246	0.226	0.213	0.233
T	0.250	0.241	0.241	0.254	0.261	0.241	0.248

- Position:

1

2

3

4

5

6

C

A

G

T

T

A

$$S_{\text{CAGTTA}} = 0.263 \times 0.296 \times 0.246 \times 0.261 \times 0.241 \times 0.263$$

$$S_{\text{CAGTTA}} = 0.000317$$

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...Example of EM - Maximization Step...

	1	2	3	4	5	6	1 ² 2 ³ 3 ⁴ 4 ⁵ 5 ⁶	RANDOM	ODDS
TCAGAA	241	230	256	226	289	263	0.000244	0.000274	0.89
CAGAAC	263	296	246	256	289	256	0.000163	0.000362	1.00
AGAACC	256	233	256	256	256	256	0.000256	0.000362	0.71
GAACCA	240	296	256	256	256	263	0.000113	0.000362	0.87
AACCAG	256	296	243	256	289	233	0.000117	0.000362	0.88
ACCAGT	256	230	243	256	213	348	0.000193	0.000274	0.71
CCAGTT	263	230	256	226	241	348	0.000209	0.000257	0.81
CAGTTA	263	296	246	261	241	263	0.000317	0.000257	1.23
AGTTAT	256	233	254	261	289	248	0.000283	0.000241	1.18
GTATAT	240	241	254	256	241	263	0.000238	0.000241	0.99
TTATAA	241	241	256	261	289	263	0.000295	0.000297	0.99
TATAAA	241	296	254	256	289	263	0.000353	0.000297	1.19
ATAAAT	256	241	256	256	289	248	0.000280	0.000318	0.91
TAAATT	241	296	256	256	241	248	0.000279	0.000297	0.94
AAATTT	256	296	256	261	241	248	0.000303	0.000297	1.02
AAATTA	256	296	254	261	241	263	0.000318	0.000297	1.07
ATTAT	256	241	254	261	289	248	0.000293	0.000278	1.05
TTATTC	241	241	254	256	241	256	0.000233	0.000278	0.84

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...Example of EM - Maximization Step...

TTATCA	241	241	256	261	256	263	0.000261	0.000297	0.88
TATCAT	241	296	254	256	289	248	0.000332	0.000297	1.12
ATCATT	256	241	243	256	241	248	0.000229	0.000297	0.77
TCATTT	241	230	256	261	241	248	0.000221	0.000278	0.80
CATTTC	263	296	254	261	241	256	0.000318	0.000297	1.07
ATTTC	256	241	254	261	256	256	0.000268	0.000297	0.90
TTTCCT	241	241	254	256	256	248	0.000240	0.000278	0.86
TTCTTT	241	241	243	256	241	248	0.000216	0.000278	0.78
TCCTTC	241	230	243	261	241	256	0.000217	0.000297	0.73
CCTTCT	263	230	254	261	256	248	0.000255	0.000297	0.86
CTTCTC	263	241	254	256	241	256	0.000254	0.000297	0.86
TTCTCC	241	241	243	261	256	256	0.000241	0.000297	0.81
TTCTCA	241	230	254	256	256	263	0.000243	0.000318	0.76
CTCCAC	263	241	243	256	289	256	0.000292	0.000339	0.86
TCCACT	241	230	243	256	256	248	0.000219	0.000318	0.69
CCACTC	263	230	256	256	241	256	0.000245	0.000339	0.72
CACCTC	263	256	243	261	256	248	0.000324	0.000339	0.95
ACTCCT	256	230	254	256	256	248	0.000243	0.000318	0.76

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...Example of EM - Maximization Step...

- The six base site **CAGTTA** beginning at base 8 is calculated to have the highest odds probability.
- Therefore, it is chosen as the new site in sequence 1.
- This is repeated for each of the sequences.
- In the maximization step, the newly chosen sites for each of the sequences are used to recalculate the frequency table.
- The expectation/maximization cycle is then repeated, until the results converge on a set of motifs.

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...Example of EM - Maximization Step

- Before:
 - Random Alignment
- TCAGAACCAAGTTATAA**ATTAT**CATTTCCTTCTCCACTCCT
- After:
 - Maximal location (given random motif alignment) (first round)
- TCAGAAC**CAGTTA**TAA**ATTAT**CATTTCCTTCTCCACTCCT

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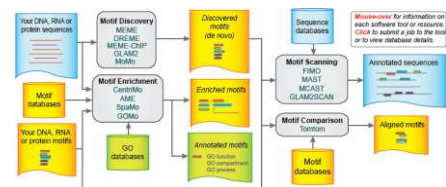
Available E-M Programs

- MEME – Uses E-M algorithms as explained
 - **Multiple EM for Motif Elicitation (MEME)** is a program developed that uses the expectation-maximization methods as described previously.
 - ParaMEME searches for blocks using the EM algorithm,
 - MetaMEME searches for profiles using Hidden Markov Models (HMMs).
- MEME locates one or more ungapped patterns in a single DNA or protein sequence, or in a series of sequences.
- A search is conducted on a variety of motif widths in order to determine the most likely width for the profile.
 - This likelihood is based on the log likelihood score calculated after the EM algorithm.

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The MEME Suite

- Motif-based sequence analysis tools



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

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MEME Software

- One of three types of motif models can be chosen:
 - **OOPS (One expected Occurrence Per Sequence)**
 - simplest model type since it assumes that there is exactly one occurrence per sequence of the motif in the dataset.
 - **ZOOPS (Zero or One expected Occurrence Per Sequence)**
 - generalization of OOPS
 - assumes zero or one motif occurrences per dataset sequence
 - **TCM (Two-Component Mixture)**
 - assumes that there are zero or more non-overlapping occurrences of the motif in each sequence in the dataset

– Bailey, Timothy L. and Charles Elkan, "The Value of Prior Knowledge in Discovering Motifs with MEME." Proceedings. International Conference on Intelligent Systems for Molecular Biology 3 (1995): 21-9.
 – https://tbailey.bitbucket.io/papers/cs95_143.pdf

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MEME Software

- Various prior knowledge can be added to MEME, including
 - the expected number of motifs,
 - the expected length of the motif,
 - whether or not the motif is palindromic
 - only applicable for DNA sequences

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Gibbs Sampling...

- Similar in nature to the EM algorithms.
 - Combines both EM and simulated annealing techniques in order to determine a maximal local alignment of multiple sequences.
 - Goal is to find most probable pattern by sampling from motif probabilities to maximize model÷background probabilities
 - The idea behind Gibbs sampling is to determine the most probable pattern common to all of the sequences by sliding them back and forth until the ratio of the motif probability to the background probability is a maximum.

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...Gibbs Sampling...

- Predictive Update Step
 - Random motif start position chosen for all sequences except one
 - Initial alignment used to calculate residue frequencies for motif and background
 - Similar to the Expectation Step of EM
- Sampling Step
 - Model probability÷background probability normalized and weighted
 - Motif start position chosen based on a random sampling with the given weights
 - Different than EM algorithm

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...Gibbs Sampling

- Process repeated until residue frequencies in each column do not change
- The sampling step is then repeated for a different initial random alignment
 - Sampling allows escape from local maxima
 - Employs a shifting routine that will take a current multiple motif alignment, and shift it a few bases to the left or the right, in order to see if only part of the motif is being found
 - A range of motif sizes can be explored in Gibbs sampling as well
- Gibbs sampling can be extended
 - to search for multiple motifs in the same set of sequences,
 - to find a pattern in only a fraction of the sequences.
- In addition, certain model-specific parameters can be enforced, such as palindromic sequences

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Hidden Markov Models...

- Hidden Markov Models (HMMs)
 - probabilistic models for studying sequences of symbols.
- HMMs can model matches, mismatches, insertions and deletions of symbols.
- HMMs have been deeply rooted in speech recognition problems.
- In speech recognition, the problem is the phonemes (or words) that have been spoken in a particular time frame.

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...Hidden Markov Models...

- Consider the difficulty.
 - Everyone you meet has a different voice.
 - Everyone speaks with a slight variation
 - this might be caused by an accent, the person having a cold, or differences in physiological development.
- However, humans are able to distinguish what the speaker is saying.
 - The idea behind speech recognition is to take in a spoken word and to try to fit it to a specific model of possible words.
 - This may in fact be close to what the brain does

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...Hidden Markov Models

- Problems in sequence analysis are similar.
- For instance,
- given an amino acid sequence, we may want to determine the protein family to which it belongs.
- The amino acid sequence can be treated similarly to the speech signal in a given frame, and the amino acids can be treated as the phonemes.

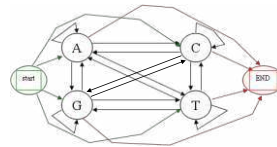
48

Markov Chain

- A probabilistic model that generates a sequence where the probability of a symbol depends upon the previous symbol.
 - A traffic light is an example of a Markov chain.
- A Markov Chain can be used to model a random DNA sequence, where there are four states:
 - A, C, G, T
 - one for each letter in the alphabet.
- When we are given a certain state, there is a transition from that state to another state with an associated probability
 - called a **transition probability**.
- An example Markov Chain can be drawn as follows:

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Markov Chain



- The key property of a Markov chain is that
 - the probability of a symbol S at position $p(S_p)$ depends only upon the previous symbol S at position $p-1(S_{p-1})$, and not on the entire previous sequence.
- Since the probability of a symbol is dependent upon the previous symbol, a prime example for the use of **Markov chains** is in the detection of **CpG islands**, which are rich in the dinucleotide **CG**.
 - **CpG (CG) islands** is a short stretch of DNA in which the frequency of the CG sequence is higher than other regions.
 - "p" simply indicates that "C" and "G" are connected by a phosphodiester bond.

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Markov Chain

- The process of methylation in biological systems will typically convert the nucleotide **C** to a **T** with a high probability when a **CG** nucleotide is encountered.
 - As a result, there will be an overabundance of the dinucleotide **TG**, and an underabundance of the dinucleotide **CG**.
- If we ignore the start and end states for now, we can see that there are sixteen different transitions.
 - A study of regions of genomic DNA has determined **normal genomic transition probabilities** to be the following.
 - where the **FROM** node is labeled along the rows to the left, and the **TO** node is labeled along the columns above:

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Markov Chain

	A	C	G	T
A	0.300	0.205	0.285	0.210
C	0.322	0.298	0.078	0.302
G	0.248	0.246	0.298	0.208
T	0.177	0.239	0.292	0.292

- The model shown above can then assign these weights to the edges of the graph

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Markov Chain

- In some regions of the genome, such as the promoter region of genes, methylation is suppressed.
 - In these regions, the dinucleotide **CG** is found in greater quantities.
- In fact, the nucleotides **C** and **G** are found to a greater degree than elsewhere in the genome.
 - A study of regions of genomic DNA where **CpG islands** exist has determined the transition probabilities to be the following:

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Markov Chain

	A	C	G	T
A	0.180	0.274	0.426	0.120
C	0.171	0.368	0.274	0.188
G	0.161	0.339	0.375	0.125
T	0.079	0.355	0.384	0.182

- A new model just like the one above can have its transition properties assigned according to the new table.
- Now we have two different models:
 - the first where **CpG** islands are absent,
 - the second where **CpG** islands are present.

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Markov Chain

- Let's call the first model the **non-CpG model** and the second model the **CpG model**.
- Given a new sequence, how would we determine whether it belongs to the **non-CpG model** or the **CpG model**?
- Remember, the key property of a Markov chain
 - the probability of a symbol **S** at position $p(S_p)$ depends only upon the previous symbol **S** at position $p_{-1}(S_{p-1})$.
 - not on the entire previous sequence.

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Markov Chain

- Therefore, to find the probability that a sequence fits a model,
 - you would multiply all of the conditional probabilities:
$$P(x) = P(x_L|x_{L-1})P(x_{L-1}|x_{L-2})\dots P(x_2|x_1)P(x_1)$$
- which can be rewritten as:

$$P(x) = P(x_1) \prod_{i=2}^L a_{x_{i-1}x_i}$$

- where $a_{x_{i-1}x_i}$ is the probability from residue at position $i-1$ to the residue at position i

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Markov Chain

- Let's consider for now that in the **non-CpG model**, $P(A) = P(T) = 0.3$; $P(C) = P(G) = 0.2$,
 - so that **A** and **T** are more probable.
- In the **CpG model**, consider $P(A) = P(C) = P(G) = P(T) = 0.25$.
- Now consider the sequence: **GGCGACG**
- The probability for this sequence:

$$P(G)P(G|G)P(C|G)P(G|C)P(A|G)P(C|A)P(G|C)$$

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Markov Chain

- For the **non-CpG model** can be calculated as:

$$(0.20)(0.298)(0.246)(0.078)(0.248)(0.205)(0.078) = 0.000000453499$$
- For the **CpG model** can be calculated as:

$$(0.25)(0.375)(0.339)(0.274)(0.161)(0.274)(0.274)(0.125) = 0.0010526$$
- Given this information, it is more likely that this sequence fits the **CpG model**.
- One thing to note is how quickly the probability gets to zero.
 - This shows the importance of using log statistics.

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Using Markov models for discrimination

- How different the **non-CpG** and **CpG models** are in relation to each other?
 - If they are not different enough, then there is not enough information to determine from which model a particular sequence is derived.
- In order to test whether we are able to discriminate between the two models, a log ratio is taken for each of the scores in the two previous tables to create a third table, where each entry, x , in the new table is equal to:

$$\log_2(P(x|\text{CpG model}) / P(x|\text{non-CpG model}))$$

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Using Markov models for discrimination

- The resulting table is as follows:

	A	C	G	T
A	-0.740	0.419	0.580	-0.803
C	-0.913	0.302	1.812	-0.685
G	-0.624	0.461	0.331	-0.730
T	-1.169	0.573	0.393	-0.679

- Using this log-odds ratio table as the scores, we can then see that
 - a sequence with a negative score will belong to the **non-CpG model**,
 - a sequence with a positive score will belong to the **CpG model**.

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Position Specific Scoring Matrix (PSSM)

- **Position Specific Scoring Matrices** incorporate information theory in order to gain a measure of how much information is contained within each column of a multiple alignment.
 - The information contained within a **PSSM** is a logarithmic transformation of the frequency of each residue in the motif.
- One problem with creating a model of a sequence alignment that is then used to search databases is that there is a bias towards the training data
 - Some residues may be underrepresented
 - Other columns may be too conserved

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Pseudocounts...

- Solution:
 - Introduce **Pseudocounts** to get a better indication
- The goal of adding **pseudocounts** is to obtain an improved estimate of the probability p_{ca} that amino acid a is in column c in all occurrences of the blocks, and not just the ones in the present sample.
- The current estimate of p_{ca} is f_{ca} , the frequency of counts in the data.
- A simplified Bayesian prediction improves the estimate of p_{ca} by adding prior information in the form of **pseudocounts**

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...Pseudocounts

- Now the estimated probability is changed from a frequency of counts in the data to the following form:

$$P_{ca} = \frac{n_{ca} + b_{ca}}{N_c + B_c}$$

- P_{ca} : Probability of residue a in column c
- n_{ca} : count of a 's in column c
- b_{ca} : pseudocount of a 's in column c
- N_c : total count in column c
- B_c : total pseudocount in column c
- These probabilities are then converted into a **log-odds** form (usually \log_2 so the information can be reported in bits) and placed in the PSSM .

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Searching PSSMs

- In order to search a sequence against a **PSSM**, the value for the first residue in the sequence occurring in the first column is calculated by searching the **PSSM**.
- Similarly, the value for the residue occurring in each column is calculated.
- These values are added (since they are logarithms) to produce a summed log odds score, S .
- This score can be converted to an odds score using the formula 2^S .
- The odds scores for the motif beginning at each position can be summed together and normalized to produce a probability of the motif occurring at each location.

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Information in PSSMs

- Information theory can give an appreciation for the amount of information contained within each sequence.
- When there is no information contained within a column, the amount of uncertainty can be measured as
 - $\log_2 20 = 4.32$ for amino acids (20 amino acids)
 - $\log_2 4 = 2$ for nucleic acid sequences (4 nucleotides)
- If only one amino acid is found in a particular column, then the uncertainty is 0 (there is only one choice).
- If there are two amino acids occurring with equal probability, then there is an uncertainty to deciding which residue it is.

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Measure of Uncertainty

- The amount of uncertainty for a particular column is measured as the **entropy**, as introduced previously

$$H_c = - \sum_{\text{residues } (a)} f_{ac} \log(p_{ac})$$

- The uncertainty for the whole **PSSM** can be calculated as a sum over all columns:

$$H_c = \sum_{\text{all columns}} H_c$$

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Relative Entropy

- In addition to the entropy measure given before, a **relative entropy** measure could be calculated as well.
 - Relative entropy takes into account not only the data in the columns of the motif, but also the overall composition of the organism being studied.
- Relative entropy can be measured as:

$$R_C = - \sum_{\text{residue}(a)} f_{ac} \log_2(p_{ac}/b_a)$$

- b_a is background frequency of residue a in the organism

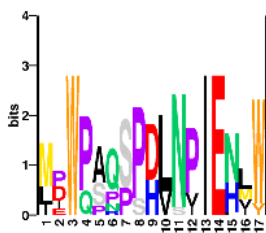
Sequence Logos...

- One way to look at a particular **PSSM** is to view it visually.
 - Sequence logos are one way to do so, by illustrating the information in each column of a motif.
- Such a graph can indicate which residues and which columns are the most important as far as sequence conservation is concerned.
 - The height of the logo is calculated as the amount by which uncertainty has been decreased
 - If the frequency in the column is less than the frequency in the background, then a negative relative entropy can be computed, which can be shown by an inverted character in the logo.

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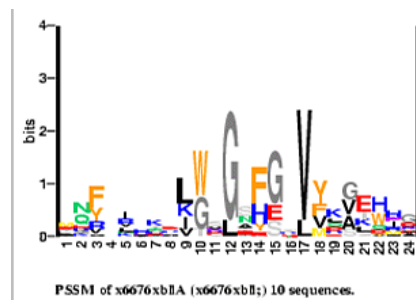
...Sequence Logos...



Logo of Gibbs Block D (Tcl) 9 sequences

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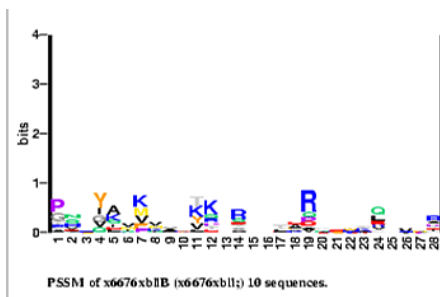
...Sequence Logos...



PSSM of x6676xb11A (x6676xb11z) 10 sequences.

70

...Sequence Logos



PSSM of x6676xb11B (x6676xb11z) 10 sequences.

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Sequence Editors

- Allow manual editing of alignments
- Add color to alignments
- Prepare images for publication
- Some sequence editors:
 - BoxShade http://www.ch.embnet.org/software/BOX_form.html
 - Serial Cloner http://serialbasics.free.fr/Serial_Cloner.html
 - GenBeans <http://www.genbeans.org/>
 - GeneStudio <http://genestudio.com/>
 - Seqtools <http://www.seqtools.dk/>
 - GENtle <http://gentle.magnusmanske.de/>
 - pDRAW32 <http://www.acaclone.com/>
 - DAMBE <http://dambe.bio.uottawa.ca/DAMBE/dambe.aspx>

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Sequence File Formats

- We have been using DNA and amino acid sequences already
- What is the typical format for these?
 - **ANSWER: Many different options**
- In order to standardize sequence data, The Nomenclature Committee of the International Union of Biochemistry and the International Union of Pure and Applied Chemistry (IUPAC) has established a standard code to represent bases that are uncertain or ambiguous.

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Standard Codes (IUPAC)

- IUPAC nucleotide codes and corresponding bases:

A = adenine	S = G or C
C = cytosine	W = A or T
G = guanine	B = G or T or C
T = thymine	D = G or A or T
U = uracil	H = A or C or T
R = G or A (purine)	V = G or C or A
Y = T or C (pyrimidine)	N = A or G or C or T (any)
K = G or T (keto)	M = A or C (amino)
M = A or C (amino)	. or - = gap
- Any other character represents an error that will not be tolerated by nearly all sequence analysis programs.

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Standard IUPAC Codes

- IUPAC standard single letter and three letter amino acid codes:

A Ala Alanine	F Phe Phenylalanine
R Arg Arginine	P Pro Proline
N Asn Asparagine	S Ser Serine
D Asp Aspartic acid	T Thr Threonine
C Cys Cysteine	W Trp Tryptophan
Q Gln Glutamine	Y Tyr Tyrosine
E Glu Glutamic acid	V Val Valine
G Gly Glycine	B Asx Aspartic acid or Asparagine
H His Histidine	Z Glx Glutamine or Glutamic acid
I Ile Isoleucine	X Xaa or Xxx Any amino acid
L Leu Leucine	
K Lys Lysine	
M Met Methionine	

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Fasta File Format

- Fasta sequence format is one of the most basic and widespread sequence formats.
- A sequence in fasta format has as its first line a descriptor beginning with a '>' character.
- The proceeding lines contain the sequence (either nucleotide or amino acid) using standard one-letter symbols.
- This format is extremely useful for sequence analysis programs, since it is devoid of numerical and non-sequence characters (with the exception of the newline character).

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Fasta File Format

- Example Fasta Sequence:

```
>gi|27819608|ref|NP_776342.1| hemoglobin, beta [beta globin] [Bos taurus]
MLTAEKAAVTAFWGKVKVDEVGGEALGRLLVVPVPTQRFESFGDLSTADAVMNNPKVKAHGKKVLDSF
SNGMKHLDDLKGTFAALSELHCDKLEVDPENFKLLGNVLVVVLARNFGKEFTPLQADFQKVAVGVANAL
AHRVH
```

- first line begins with '>', followed by **gi**,
 - next field surrounded by '|' is GenBank identifier
- the keyword 'ref'
 - field will be the reference for the version of this sequence.
- final field is the description

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Fasta File Format

- Example Fasta Sequence:

```
>gi|27819608|ref|NP_776342.1| hemoglobin, beta [beta globin] [Bos taurus]
MLTAEKAAVTAFWGKVKVDEVGGEALGRLLVVPVPTQRFESFGDLSTADAVMNNPKVKAHGKKVLDSF
SNGMKHLDDLKGTFAALSELHCDKLEVDPENFKLLGNVLVVVLARNFGKEFTPLQADFQKVAVGVANAL
AHRVH
```

- nearly all sequence based programs treat anything following the '>' as a comment
- a few sequence analysis programs expect sequences to be in a strict fasta format

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GenBank

- GenBank is the National Center for Biotechnology Information’s nucleic acid and protein sequence database.
- It is the most widely used source of biological sequence data.
- GenBank file format contains information about the sequence, including literature references, functions of the sequence, locations of various features, etc.
- information organized into fields, each with an identifier, justified to the farthest left column.
- Some identifiers have additional subfields.
- sequence data lies between the identifier ORIGIN and the “//” which signals the end of a GenBank record.

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GenBank Record

LOCUS HBB 145 aa linear MAM 22-JAN-2003
DEFINITION hemoglobin, beta [beta globin] [Bos taurus].
ACCESSION NP_776342
VERSION NP_776342.1 GI:27819608
DBSOURCE REFSEQ: accession [NM_173917.1](#)
KEYWORDS .
SOURCE Bos taurus (cow)
ORGANISM [Bos taurus](#); Eukaryota; Metazoa; Chordata; Craniata;
Vertebrata; Euteleostomi; Mammalia; Eutheria; Cetartiodactyla;
Ruminantia; Pecora; Bovidae; Bovinae; Bos.
REFERENCE 1 (residues 1 to 145)
AUTHORS Duncan,C.H.
JOURNAL Unpublished (1991)
COMMENT PROVISIONAL [REFSEQ](#): This record has not yet been subject to final
NCBI review. The reference sequence was derived from [MG3453.1](#).
FEATURES Location/Qualifiers source 1..145

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ASN.1

- Abstract Syntax Notation (ASN.1):
 - formal description language developed to encode various data to be easily connected across computer systems
- ASN.1 is highly structured and detailed
- ASN.1 format contains all of the other information found in other formats

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