Lecture 12
Regulatory Motifs: Representation, Discovery, and instance identification

Challenges in Computational Biology

Motif discovery overview
1. Introduction to regulatory motifs / gene regulation
   - Two settings: co-regulated genes (EM,Gibbs), de novo
2. Expectation maximization: Motif matrix \( \mathbf{Z} \) positions
   - E step: Estimate motif positions \( \mathbf{Z} \) from motif matrix
   - M step: Find max-likelihood motif from all positions \( \mathbf{Z} \)
3. Gibbs Sampling: Sample from joint \( (\mathbf{M},\mathbf{Z}) \) distribution
   - Sampling motif positions based on the \( \mathbf{Z} \) vector
   - More likely to find global maximum, easy to implement
4. Evolutionary signatures for de novo motif discovery
   - Genome-wide conservation scores, motif extension
   - Validation of discovered motifs: functional datasets
5. Evolutionary signatures for instance identification
   - Phylogenies, Branch length score \( \rightarrow \) Confidence score
   - Foreground vs. background. Real vs. control motifs.

The regulatory code: All about regulatory motifs

- The parts list: \( \sim 20-30k \) genes
  - Protein-coding genes, RNA genes (rRNA, microRNA, snRNA)
- The circuitry: constructs controlling gene usage
  - Enhancers, promoters, splicing, post-transcriptional motifs
- The regulatory code, complications:
  - Combinatorial coding of ‘unique tags’
    - Data-centric encoding of addresses
  - Overlaid with ‘memory’ marks
    - Large-scale on/off states
  - Modulation of the large-scale coding
- Today: discovering motifs in co-regulated promoters and de novo motif discovery & target identification

Regulatory motif discovery

- Regulatory motifs
  - Genes are turned on / off in response to changing environments
  - No direct addressing: subroutines (genes) contain sequence tags (motifs)
  - Specialized proteins (transcription factors) recognize these tags

- What makes motif discovery hard?
  - Motifs are short (6-8 bp), sometimes degenerate
  - Can contain any set of nucleotides (no ATG or other rules)
  - Act at variable distances upstream (or downstream) of target gene
How Transcription Factors actually recognize motifs

- Proteins ‘feel’ DNA
  - Read chemical properties of bases
  - Do NOT open DNA (no base complementarity)
- 3D Topology dictates specificity
  - Fully constrained positions: every atom matters
  - ‘Ambiguous / degenerate’ positions loosely contacted
- Other types of recognition
  - MicroRNAs: complementarity
  - Nucleosomes: GC content
  - RNAs: structure/seqn combination

Motifs summarize TF sequence specificity

- Summarize information
- Integrate many positions
- Measure of information
- Distinguish motif vs. motif instance
- Assumptions:
  - Independence
  - Fixed spacing

Experimental factor-centric discovery of motifs

SELEX (Systematic Evolution of Ligands by Exponential Enrichment; Klug & Famulok, 1994)
DIP-Chip (DNA-immunoprecipitation with microarray detection; Liu et al., 2005)
PBMs (Protein binding microarrays; Mukherjee, 2004) Double stranded DNA arrays

Challenges in regulatory genomics

TFs: Homology to TFs/domains
miRNAs: Evolutionary signatures
miRNAs: Experimental cloning

TFs: SELEX, DIP-Chip, Protein-Binding-Microarrays
miRNAs: Evolutionary/Structural signatures
miRNAs: Experimental cloning of 5’ ends

TFs: Mass Spec (difficult)
TFs/miRNAs: De novo comparative discovery**

Regulator
TFs/motifs
Motif
Y
Sequence
specifity
Targets
Network analysis (next lecture)
TFs: ChIP-Chip/ChIP-Seq
TFs/miRNAs: Perturbation response

TFs/miRNAs: Evolutionary signatures**
miRNAs: Composition/Folding

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How would you go about it?

Given a set of co-regulated/functionally related genes, find common motifs in their promoter regions

- Align the promoters to each other using local alignment
- Use expert knowledge for what motifs should look like
- Find ‘median’ string by enumeration (motif/sample driven)
- Start with conserved blocks in the upstream regions
Motifs are not limited to DNA sequences

- Splicing Signals at the RNA level
  - Splice junctions
  - Exonic Splicing Enhancers (ESE)
  - Exonic Splicing Suppressors (ESS)
- Domains and epitopes at the Protein level
  - Glycosylation sites
  - Kinase targets
  - Targetting signals
  - MHC binding specificities
- Recurring patterns at the physiological level
  - Expression patterns during the cell cycle
  - Heart beat patterns predicting cardiac arrest
  - Final project in previous year, now used in Boston hospitals!

### Starting positions ⇔ Motif matrix

- given **aligned** sequences → easy to compute profile matrix

#### shared motif

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
<td>0.6</td>
<td>0.1</td>
<td>0.5</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>G</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6</td>
<td>0.5</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>T</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

#### sequence positions

- easy to find starting position probabilities

**Key idea:** Iterative procedure for estimating both, given uncertainty

(learning problem with hidden variables: the starting positions)

### Basic Iterative Approach

Given: length parameter \( W \), training set of sequences

- set initial values for motif
- do
  - re-estimate starting-positions from motif
  - re-estimate motif from starting-positions
- until convergence (change < \( \epsilon \))
- return: motif, starting-positions

### Representing Motif \( M(k,c) \) and Background \( B(c) \)

- Assume motif has fixed width, \( W \)
- Motif represented by matrix of probabilities: \( M(k,c) \)
  - the probability of character \( c \) in column \( k \)

\[
\begin{array}{cccc}
A & 0.1 & 0.5 & 0.2 \\
C & 0.4 & 0.2 & 0.1 \\
G & 0.3 & 0.1 & 0.6 \\
T & 0.2 & 0.2 & 0.1 \\
\end{array}
\]

- Background represented by \( B(c) \), frequency of each base

\[
\begin{array}{cccc}
A & 0.26 \\
C & 0.24 \\
G & 0.23 \\
T & 0.27 \\
\end{array}
\]

### Representing the starting position probabilities \( Z_{ij} \)

- the element \( Z_{ij} \) of the matrix \( Z \) represents the probability that the motif starts in position \( j \) in sequence \( i \)

\[
Z = \begin{bmatrix}
0.1 & 0.1 & 0.2 & 0.6 \\
0.4 & 0.2 & 0.1 & 0.3 \\
0.3 & 0.1 & 0.5 & 0.1 \\
0.1 & 0.5 & 0.1 & 0.3 \\
\end{bmatrix}
\]

Some examples:

- \( Z_1 \): Probability that on sequence 1, motif start at position 1
- \( Z_2 \): Probability that on sequence 2, motif start at position 2
- \( Z_3 \): Probability that on sequence 3, motif start at position 3
- \( Z_4 \): Probability that on sequence 4, motif start at position 4

### Starting positions \( Z_{ij} \) ⇔ Motif matrix \( M(k,c) \)

- given \( Z_{ij} \) matrix from \( M(k,c) \)
  - easy to re-compute \( M(k,c) \) from \( Z_{ij} \) matrix

- Expectation maximization
  - All starts weighted by \( Z_{ij} \) prob distribution
- Gibbs sampling
  - Single start for each seq \( X_i \) by sampling \( Z_{ij} \)
- Greedy approach
  - Best start for each seq \( X_i \) by maximum \( Z_{ij} \)

- Three variations for re-computing motif \( M(k,c) \) from \( Z_{ij} \) matrix

- M-step
- E-step

- Motif: \( M(k,c) \)
- Starting positions: \( Z_{ij} \)
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Three examples for Greedy, Gibbs Sampling, EM

- **Greedy always picks maximum**
- **Gibbs sampling picks one at random**
- **EM uses both in estimating motif**

Calculating the Z vector (using M)
- To estimate the starting positions in Z at step \(t\) likelihood
  \(Z_{i(t)} = \Pr(Z_i = 1 | X, M^{(t)}) = \frac{\Pr(X_i | Z_i = 1, M^{(t)}) \Pr(Z_i = 1)}{\Pr(X_i)}\) (Bayes’ rule)
- At iteration \(t\), calculate \(Z_{i(t)}\) based on \(M^{(t)}\)
  - We just saw how to calculate \(\Pr(X_i | Z_i = 1, M^{(t)})\)
  - To obtain total probability \(\Pr(X_i)\), sum over all starting positions
    \(Z_{i(t)} = \frac{\Pr(X_i | Z_i = 1, M^{(t)}) \Pr(Z_i = 1)}{\sum_{i} \Pr(X_i | Z_i = 1, M^{(t)}) \Pr(Z_i = 1)}\)
  - Assume uniform priors (motif eq likely to start at any position)

Calculating \(\Pr(X_i)\) when motif position is known
- Probability of training sequence \(X_i\) given hypothesized start position \(j\)
  \(\Pr(X_i | Z_i = 1, M, B) = \prod_{j=1}^{L} B(X_{ij}) \prod_{j=1}^{L} M(k - j + 1, X_{ij}) \prod_{j=1}^{L} B(X_{ij} \backslash k)\)

- Example:
  \(X_i = G\ C\ T\ G\ T\ A\ G\ \ B = A\ 0.25\ C\ 0.25\ G\ 0.25\ T\ 0.25\ \ M = \begin{bmatrix} 1 & 1 & 2 & 3 \\ 0.1 & 0.2 & 0.2 & 0.1 \\ 0.3 & 0.1 & 0.2 & 0.1 \end{bmatrix}\)
  \(\Pr(X_i | Z_i = 1, M, B) = B(G) \times B(C) \times M(1, T) \times M(2, G) \times M(3, T) \times B(A) \times B(G) = 0.25 \times 0.25 \times 0.25 \times 0.25 \times 0.25 \times 0.25 \times 0.25\)

Calculating the Z vector: Example
- \(X_i = G\ C\ T\ G\ T\ A\ G\ \ P = \begin{bmatrix} 0 & 1 & 2 & 3 \\ A & 0.25 & 0.3 & 0.5 & 0.2 \\ C & 0.25 & 0.4 & 0.2 & 0.1 \\ G & 0.25 & 0.3 & 0.1 & 0.6 \\ T & 0.25 & 0.2 & 0.2 & 0.1 \end{bmatrix}\)
- \(Z_{i(t)} = \begin{bmatrix} 0.3 \times 0.2 \times 0.1 & 0.25 \times 0.25 \times 0.25 \times 0.25 \\ 0.25 \times 0.4 \times 0.2 \times 0.6 \times 0.25 \times 0.25 \times 0.25 \times 0.25 \end{bmatrix}\)
- then normalize so that \(\sum_{j=1}^{L} Z_{ij} = 1\)
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#### The M-step: Estimating the motif \(M\)

- \(\text{recall } M(k,c)\) represents the probability of character \(c\) in position \(k\), \(B(c)\) stores values for the background

\[
M^{(t+1)}(k,c) = \frac{n_{k,c} + d}{\sum_c (n_{k,c} + d)} \quad \text{pseudo-counts}
\]

- \(B^{(t+1)}(c) = \frac{n_{0,c} + d}{\sum_c (n_{0,c} + d)} \quad \text{where } n_{0,c} = n_{c} - \sum_{j=1}^{W} n_{j,c}\)

---

#### The EM Algorithm

- EM converges to a local maximum in the likelihood of the data given the model:

\[
\prod_l \Pr(X_l | \theta) = \int \Pr(X_l | M,B) \Phi(M,B) dM dB
\]

- Deterministic iterations max direction of ascent
- Usually converges in a small number of iterations
- Sensitive to initial starting point (i.e. values in \(M\))

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#### M-step example: Estimating \(M(k,c)\) from \(Z\)

\[
\begin{align*}
X_1 &= A \quad C \quad C \quad A \\
Z_1 &= 0.1 \quad 0.7 \quad 0.1 \quad 0.1 \\
X_2 &= A \quad G \quad G \quad C \quad A \\
Z_2 &= 0.4 \quad 0.1 \quad 0.1 \quad 0.4 \\
X_3 &= T \quad C \quad A \quad G \\
Z_3 &= 0.2 \quad 0.6 \quad 0.1 \quad 0.1 \\
\end{align*}
\]

- EM: sum over full probability
  - \(n_{k,c} = 0.1\times0.4\times0.1 = 0.7\)
  - \(n_{k,c} = 0.7\times0.4\times0.6 = 1.7\)
  - \(n_{k,c} = 0.1\times0.4\times0.1 = 0.4\)
  - \(n_{k,c} = 0.2 \times 0.2 = 0.2\)
  - Total: \(0.7+1.7=0.4+0.2 = 3.0\)

- Normalize and add pseudo-counts
  - \(M(1,A) = (0.7+1)(T+A) = 1.7/7=0.24\)
  - \(M(1,C) = (1.7+1)(T+C) = 2.7/7=0.39\)
  - \(M(1,G) = (0.4+1)(T+G) = 1.4/7=0.2\)
  - \(M(1,T) = (0.2+1)(T+G) = 1.2/7=0.17\)

- \(M(k,c) = \frac{\sum_{j=1}^{W} Z_{k,j} + Z_{c,j} + 1}{\sum_{j=1}^{W} Z_{k,j} + Z_{c,j} + 4}\)

\[
\begin{array}{|c|c|c|c|}
\hline
\text{EM approach: Avg' em all} & \text{Gibbs sampling: Sample one} & \text{Greedy: Select max} \\
\hline
\text{Avg'.em all} & \text{Gibbs sampling} & \text{Greedy} \\
\hline
\text{M(k,c)} & \text{M(k,c)} & \text{M(k,c)} \\
\hline
0.29 & 0.41 & 0.4 \\
0.39 & 0.24 & 0.66 \\
0.17 & 0.98 & 0.98 \\
\hline
\end{array}
\]
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Three examples of Greedy, Gibbs Sampling, EM

Greedy always picks maximum

Gibbs sampling picks one at random

\((\mathbf{Z}_1)\)

EM uses both in estimating motif

All methods agree

EM averages over the entire sequence (no preference)

Gibbs sampling rapidly converges to some choice

Greedy ignores most of the probability

Three options for assigning points, and their parallels across K-means, HMMs, Motifs

<table>
<thead>
<tr>
<th>Update rule</th>
<th>Update assignments (E step)</th>
<th>Algorithm implementing E step</th>
<th>Update model parameters (M step)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick one label at random, based on their relative probability</td>
<td>Assign each point to best label</td>
<td>(\text{K-means: Assign each point to nearest cluster})</td>
<td>Average of those points assigned to label</td>
</tr>
<tr>
<td>Pick one label at random, according to posterior probability</td>
<td>Assign each point to all labels, probabilistically</td>
<td>(\text{Fuzzy K-means: Assign to all clusters, weighted by proximity})</td>
<td>Average of all points, weighted by membership</td>
</tr>
<tr>
<td>Pick one label at random, according to match score</td>
<td>N/A: Assign to a random cluster, sample by proximity</td>
<td>(\text{Baum-Welch training: label sequences with posterior decoding})</td>
<td>Gibbs sampling: Use one position for the motif, by sampling from the match score</td>
</tr>
</tbody>
</table>

Gibbs Sampling

- A general procedure for sampling from the joint distribution of a set of random variables \(\Pr(U_1, \ldots, U_n)\) by iteratively sampling from each \(\Pr(U_j | U_1, \ldots, U_{j-1}, U_{j+1}, \ldots, U_n)\)
- Useful when it’s hard to explicitly express means, stdevs, covariances across the multiple dimensions
- Useful for supervised, unsupervised, semi-supervised learning
  - Specify variables that are known, sample over all other variables
- Approximate:
  - Joint distribution: the samples drawn
  - Marginal distributions: examine samples for subset of variables
  - Expected value: average over samples
- Example of Markov-Chain Monte Carlo (MCMC)
  - The sample approximates an unknown distribution
  - Stationary distribution of sample (only start counting after burn-in)
  - Assume independence of samples (only consider every 100)
- Special case of Metropolis-Hastings
  - In its basic implementation of sampling step
  - But it’s a more general sampling framework
**Gibbs Sampling for motif discovery**

- First application to motif finding: Lawrence et al 1993
- Can view as a stochastic analog of EM for motif discovery task
- Less susceptible to local minima than EM
- EM maintains distribution $Z_i$ over the starting points for each seq
- Gibbs sampling selects specific starting point $a_i$ for each seq → but keeps resampling these starting points

**Given:** length parameter $W$, training set of sequences $X_1, ..., X_N$

**Choose random positions for $a$**

**Do**

Pick a sequence $X_i$

Estimate $p$ given current motif positions $a$ (update step)

Sample a new motif position $a_i$ for $X_i$ (sampling step)

Until convergence

Return: $p, a$

---

**Popular implementation: AlignACE, BioProspector**

AlignACE: first statistical motif finder
BioProspector: improved version of AlignACE

Both use basic Gibbs Sampling algorithm:

1. **Initialization**:
   - Select random locations in sequences $X_1, ..., X_N$
   - Compute an initial model $M$ from these locations

2. **Sampling Iterations**:
   - Remove one sequence $X_i$
   - Recalculate model
   - Pick a new location of motif in $X_i$ according to probability the location is a motif occurrence

In practice, run algorithm from multiple random initializations:

1. Initialize
2. Run until convergence
3. Repeat 1,2 several times, report common motifs

---

**Gibbs Sampling (AlignACE)**

**Predictive Update**

- Select a sequence $X_i$
- Remove $X_i$, recompute model:

$$M(k, c) = \frac{d + \sum_{j=1}^{W-1} (X_{i,j + k} = c)}{(N - 1) + 4d}$$

where $d$ is a pseudocount to avoid $0$s

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**Sampling New Motif Positions**

- For each possible starting position, $a_j$, compute a weight

$$A_j = \prod_{k=2}^{W-1} \frac{M(k-j+1, X_{i,k})}{B(X_{i,k})}$$

- Randomly select a new starting position $a_i$ according to these weights (normalizing across the sequence, again like with MEME)

- Note, this is equivalent to using the likelihood from MEME because:

$$A_j \propto \Pr(X_i | Z_{ij} = 1, p)$$

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**Advantages / Disadvantages**

- Very similar to EM

**Advantages**:  
- Easier to implement  
- Less dependent on initial parameters  
- More versatile, easier to enhance with heuristics

**Disadvantages**:  
- More dependent on all sequences to exhibit the motif  
- Less systematic search of initial parameter space
Motivation for de novo genome-wide motif discovery

- Both TF and region centric approaches are not comprehensive and are biased
- TF centric approaches generally require transcription factor (or antibody to factor)
  - Lots of time and money
  - Also have computational challenges
- De novo discovery using conservation is unbiased but can’t match motif to factor and require multiple genomes

Genome-wide conservation

Evaluate conservation within:

<table>
<thead>
<tr>
<th></th>
<th>Gal4</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) All intergenic regions</td>
<td>13%</td>
<td>2%</td>
</tr>
<tr>
<td>(2) Intergen : coding</td>
<td>13% : 3%</td>
<td>2% : 7%</td>
</tr>
<tr>
<td>(3) Upstream : downstream</td>
<td>12:0</td>
<td>1:1</td>
</tr>
</tbody>
</table>

A signature for regulatory motifs

Conservation islands overlap known motifs

<table>
<thead>
<tr>
<th>Known engrailed binding site</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. melanogaster (D.m.)</td>
</tr>
<tr>
<td>D. simulans (D.s.)</td>
</tr>
<tr>
<td>D. yakuba (D.y)</td>
</tr>
<tr>
<td>D. erecta (D.e)</td>
</tr>
<tr>
<td>D. ananassae (D.a)</td>
</tr>
</tbody>
</table>

- Start by looking at known motif instances
- Individual motif instances are preferentially conserved
- Can we just take conservation islands and call them motifs?
  - No. Many conservation islands are due to chance or perhaps due to non-motif conservation

Evolutionary signatures for regulatory motifs

- Phylogenies, Branch length score
- Foreground vs. background. Real vs. control motifs.

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Gibbs Sampling and Climbing

Because gibbs sampling does always choose the best new location it can move to another place not directly uphill

In theory, Gibbs Sampling less likely to get stuck a local maxima

<table>
<thead>
<tr>
<th>Conservation islands overlap known motifs</th>
</tr>
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<tbody>
<tr>
<td>Spat</td>
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</tbody>
</table>

Increase power by testing conservation in many regions
**Conservation for TF motif discovery**

1. Enumerate motif seeds
   
   \[
   \text{T G C \quad \text{gap} \quad \text{T A G}}
   \]
   
   • Six non-degenerate characters with variable size gap in the middle

2. Score seed motifs
   
   • Use a conservation ratio corrected for composition and small counts to rank seed motifs

3. Expand seed motifs
   
   \[
   \text{S R T G C Y \quad \text{gap} \quad W T A G R}
   \]
   
   • Use expanded nucleotide IUPAC alphabet to fill unspecified bases around seed using hill climbing

4. Cluster to remove redundancy
   
   • Using sequence similarity

---

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4. Evolutionary signatures for de novo motif discovery
   
   - Genome-wide conservation scores, motif extension
   - Validation of discovered motifs: functional datasets

5. Evolutionary signatures for instance identification
   
   - Phylogenies, Branch length score \( \rightarrow \) Confidence score
   - Foreground vs. background. Real vs. control motifs.

---

**Learning motif degeneracy using evolution**

- Record frequency with which one sequence is "replaced" by another in evolution
- Use this to find clusters of k-mers that correspond to a single motif

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**Test 1: Intergenic conservation**

- CGG-11-CCG

**Test 2: Intergenic vs. Coding**

- CGG-11-CCG

**Test 3: Upstream vs. Downstream**

- CGG-11-CCG

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**Kellis, Nature 2003**

**Tanay, Genome Research 2004**
Validation of the discovered motifs

- Because genome-wide motif discovery is de novo, we can use functional datasets for validation
  - Enrichment in co-regulated genes
  - Overlap with TF binding experiments
  - Enrichment in genes from the same complex
  - Positional biases with respect to transcription start
  - Upstream vs. downstream / inter vs. intra-genic bias
  - Similarity to known transcription factor motifs
- Each of these metrics can also be used for discovery
  - In general, split metrics into discovery vs. validation
  - As long as they are independent!
  - Strategies that combine them all lose ability to validate
- Directed experimental validation approaches are then needed

Similarity to known motifs

- If discovered motifs are real, we expect them to match motifs in large databases of known motifs
- We find this (significantly higher than with random motifs)
- Why not perfect agreement?
  - Many known motifs are not conserved
  - Known motifs are biased; may have missed real motifs

<table>
<thead>
<tr>
<th>MCS</th>
<th>Known Factor</th>
<th>Discovered Motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>65.6</td>
<td>en</td>
<td>CTATTAAA</td>
</tr>
<tr>
<td>57.3</td>
<td>repo</td>
<td>TTKCAATTAA</td>
</tr>
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<td>54.9</td>
<td>ara</td>
<td>WATTRATTK</td>
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<td>54.4</td>
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<tr>
<td>37.6</td>
<td>KAPPA</td>
<td>TACTCAAC</td>
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</table>

MCS = Discovered motif

<table>
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<tr>
<th>MCS</th>
<th>Known Factor</th>
<th>Discovered Motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>70/174</td>
<td>mammalian motifs</td>
<td></td>
</tr>
</tbody>
</table>

Positional bias of motif matches

- Motifs are involved in initiation of transcription
  - Motif matches biased versus TSS
    - 10% of fly motifs
    - 34% of mammalian motifs
  - Depletion of TF motifs in coding sequence
    - 57% of fly motifs
  - Clustering of motif matches
    - 19% of fly motifs

Motifs have functional enrichments

For both fly (top) and mammals (bottom), motifs are enriched in genes expressed in specific tissues

Reveals modules of cooperating motifs

Experimental target identification: ChIP-chip/seq

Limitations:
- Antibody availability
- Restricted to specific stages/tissues
- Biological functionality of most binding sites unknown
- Resolution can be limited (can’t usually identify the precise base pairs)

Kheradpour, Stark, Roy, Kellis, Genome Research 2007
Computational target identification

- Single genome approaches using motif clustering (e.g. Berman 2002; Schroeder 2004; Philippakis 2006)
  - Requires set of specific factors that act together
  - Miss instances of motifs that may occur alone
- Multi-genome approaches (phylogenetic footprinting) (e.g. Moses 2004; Blanchette and Tompa 2002; Etwiller 2005; Lewis 2003)
  - Tend to either require absolute conservation or have a strict model of evolution

Challenges in target identification

- Simple case
  - Instance fully conserved in orthologous position near genes
- Motif turn-around/movement
  - Motif instance is not found in orthologous place due to birth/death or alignment errors
- Distal/missing matches
  - Due to sequencing/assembly errors or turnover
  - Distal instances can be difficult to assign to gene

Computing Branch Length Score (BLS)

CF

Branch Length Score ➔ Confidence

1. Evaluate chance likelihood of a given score
   - Sequence could also be conserved due to overlap with un-annotated element (e.g. non-coding RNA)
2. Account for differences in motif composition and length
   - For example, short motif more likely to be conserved by chance

Producing control motifs

- When evaluating the conservation, enrichment, etc. of motifs, it is useful to have a set of “control motifs

  1. Produce 100 shuffles of our original motif
  2. Filter motifs, requiring they match the genome with about (+/- 20%) of our original motif
  3. Sort potential control motifs based on their similarity to other known motifs
  4. Cluster potential control motifs and take at most one from each cluster, in increasing order of similarity to known motifs

  branch length score
  confidence

  1. Use motif-specific shuffled control motifs determine the expected number of instances at each BLS by chance alone or due to non-motif conservation
  2. Compute Confidence Score as fraction of instances over noise at a given BLS (+1 – false discovery rate)
Computing enrichments: background vs. foreground

- Background vs. foreground
  - co-regulated promoters vs. all genes
  - Bound by TF vs. other intergenic regions
- Enrichment: fraction of motif instances in foreground vs. fraction of bases in foreground
- Correct for composition/conservation level: compute enrichments w/ control motifs
  - Fraction of motif instances can be compared to fraction of control motif instances in foreground
  - A hypergeometric p-value can be computed (similar to $\chi^2$, but better for small numbers)
- Fractions can be made more conservative using a binomial confidence interval

**Validation of discovered motif instances**

Use independent experimental evidence
Look for functional biases / enrichments

**Increased sensitivity using BLS**

![Graphs showing sensitivity improvements with BLS](image)

**Intersection with CTCF ChIP-Seq regions**

ChIP-Seq and ChIP-Chip technologies allow for identifying binding sites of a motif experimentally
- Conserved CTCF motif instances highly enriched in ChIP-Seq sites

**Confidence selects for functional instances**

1. Confidence selects for transcription factor motif instances in promoters and miRNA motifs in 3' UTRs
2. miRNA motifs are found preferentially on the plus strand, whereas no such preference is found for TF motifs
Enrichment found for many factors

Barski, et al., Cell (2007)


Lim, et al., Molecular Cell (2007)

Wei, et al., Cell (2006)


Abrams and Andrew, Devel (2005) (Not ChIP)


Zeitlinger, et al., Genes & Devel (2007)

Sandmann, et al., Genes & Devel (2007)

Enrichment increases in conserved bound regions

1. ChIP bound regions may not be conserved
2. For CTCF we also have binding data in mouse
3. Enrichment in intersection is dramatically higher

More enrichment when binding conserved

1. ChIP bound regions may not be conserved
2. For CTCF we also have binding data in mouse
3. Enrichment in intersection is dramatically higher
4. Trend persists for other factors where we have multi-species ChIP data

Comparing ChIP to Conservation

1. Motifs at 60% confidence and ChIP have similar enrichments (depletion for the repressor Snail) in the functional promoters
2. Enrichments persist even when you look at non-overlapping subsets
3. Intersection of two regions has strongest signal
4. Evolutionary and experimental evidence is complementary
   - ChIP includes species different regions and different tissues
   - Conserved instances include binding sites not seen in tissues surveyed

Fly regulatory network at 60% confidence

TFs: 67 of 83 (81%) 46k instances

miRNAs: 49 of 67 (86%) 4k instances

Several connections confirmed by literature (directly or indirectly)

Global view of instances allows us to make network level observations:
- 46% of targets were co-expressed with their factor in at least one tissue (P < 2 x 10^-5)
- TFs were more targeted by TFs (P < 1 x 10^-5) and by miRNAs (P < 3 x 10^-5).
- TF in-degree associated with miRNA in-degree (high-high: P < 10^-5; low-low P < 10^-7)

Motif discovery overview

1. Introduction to regulatory motifs / gene regulation
   - Two settings: co-regulated genes (EM,Gibbs), de novo
2. Expectation maximization: Motif matrix positions
   - E step: Estimate motif positions \( Z_i \) from motif matrix
   - M step: Find max-likelihood motif from all positions \( Z_i \)
3. Gibbs Sampling: Sample from joint (M,Z) distribution
   - Sampling motif positions based on the Z vector
   - More likely to find global maximum, easy to implement
4. Evolutionary signatures for de novo motif discovery
   - Genome-wide conservation scores, motif extension
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