Inferring Models of cis-Regulatory Modules using Information Theory

BMI/CS 776
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Overview

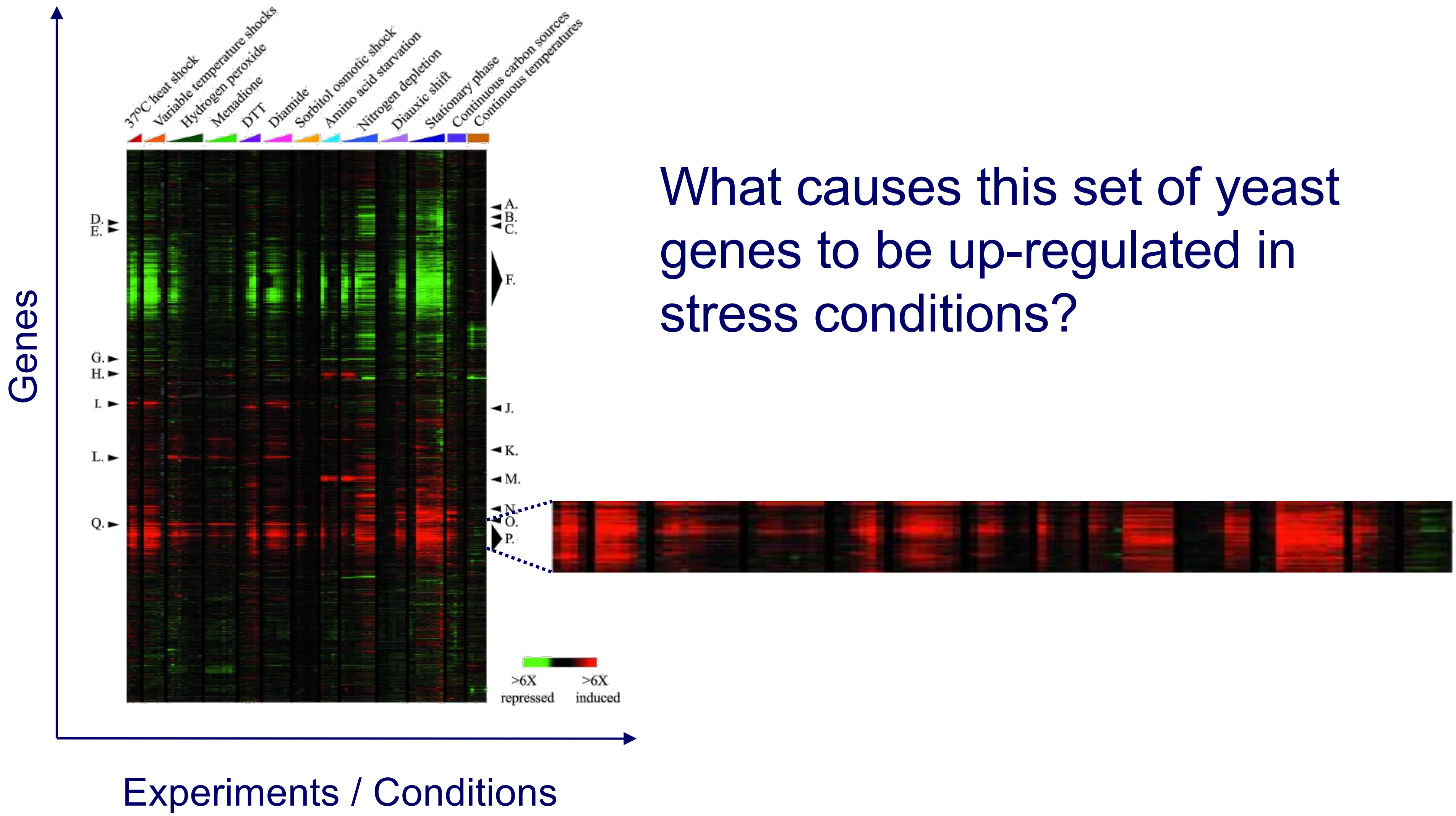
- Biological question
  - What is causing differential gene expression?
- Goal
  - Find regulatory motifs in the DNA sequence
- Solution
  - FIRE (Finding Informative Regulatory Elements)

Goals for Lecture

Key concepts:

- Entropy
- Mutual information (MI)
- Motif logos
- Using MI to identify cis-regulatory module elements

A Common Type of Question

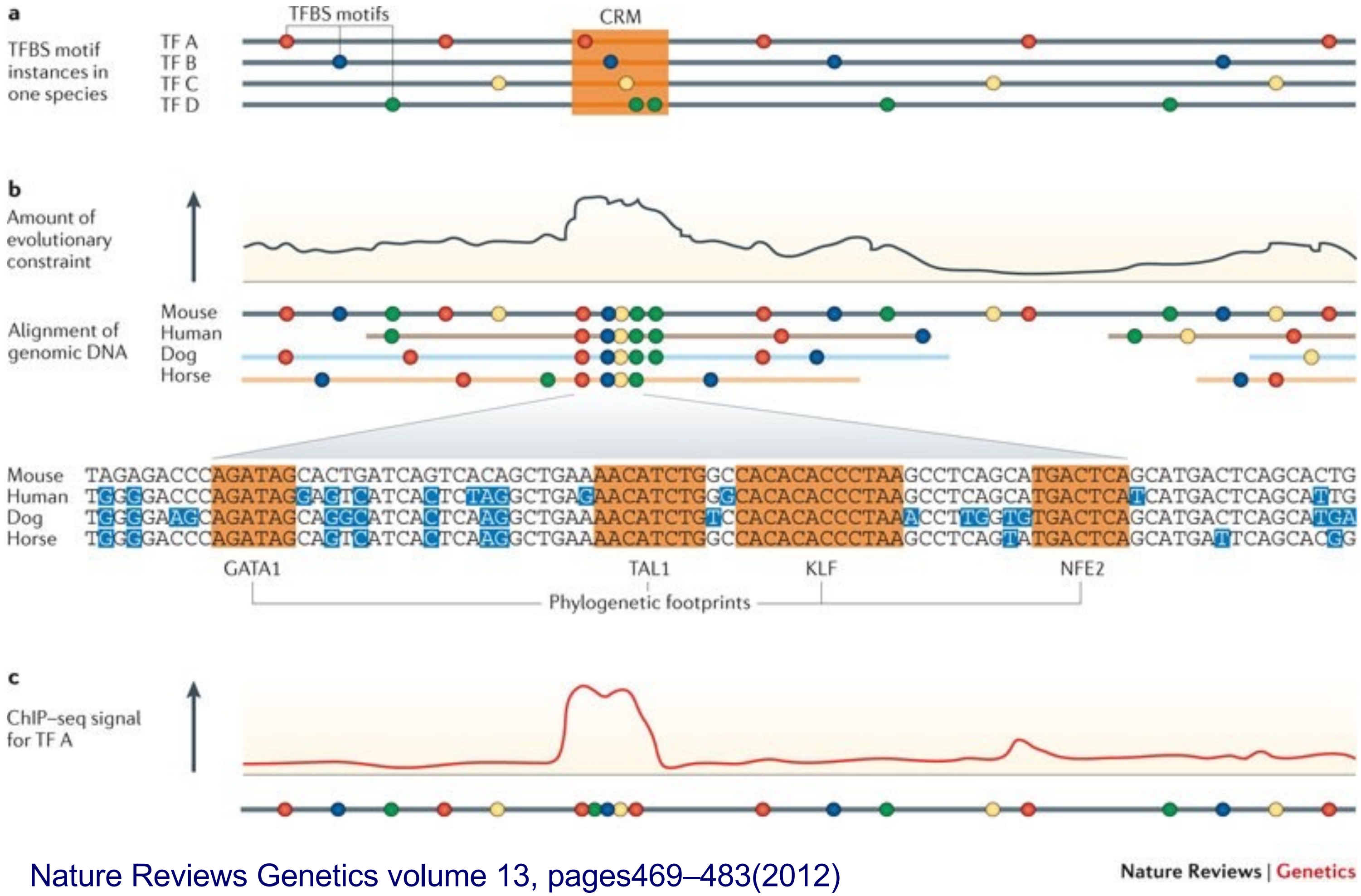


What causes this set of yeast genes to be up-regulated in stress conditions?

Figure from Gasch *et al.*, *Mol. Biol. Cell*, 2000

cis-Regulatory Modules (CRMs)

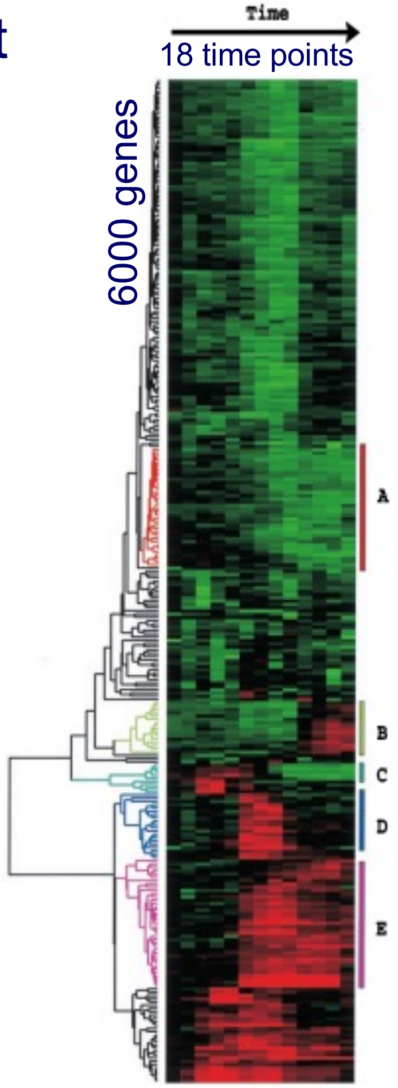
- Co-expressed genes are often controlled by specific configurations of binding sites



Nature Reviews Genetics volume 13, pages469–483(2012)

Co-expressed genes have similar functions in single species

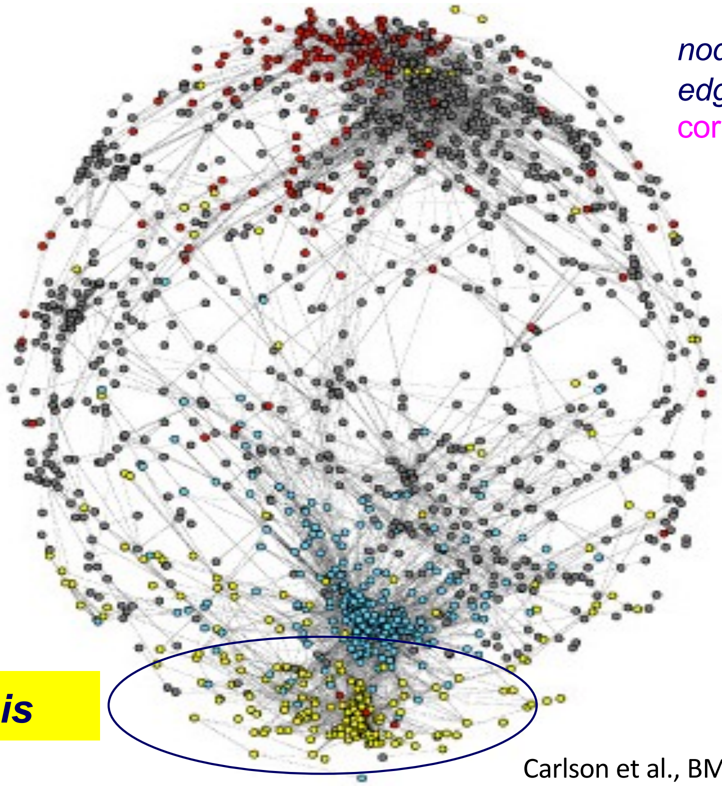
Yeast
cell
cycle



Eisen et al., PNAS, 1998.

A gene co-expression network (relationship) can reveal ***functional groupings***

- Hierarchal clustering, K-means, Gaussian mixture model (GMM), Principal component analysis (PCA), …



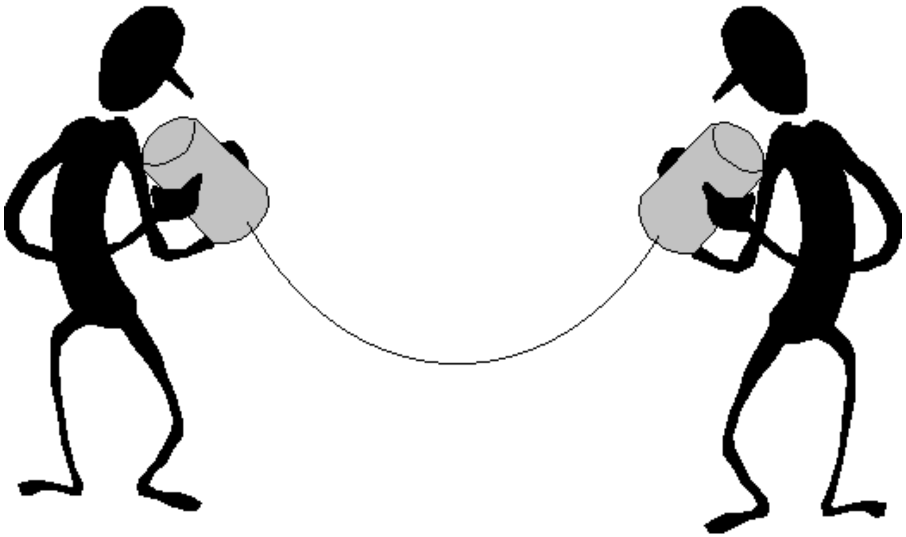
node: gene
edge: expression correlation

Protein synthesis

Carlson et al., BMC Bioinformatics, 2006.

Information Theory Background

- Problem
  - Create a code to communicate information
- Example
  - Need to communicate the manufacturer of each bike



Information Theory Background

- Four types of bikes
- Possible code

Type	code
Trek	11
Specialized	10
Cervelo	01
Serotta	00

- Expected number of bits we have to communicate: 2 bits/bike

Information Theory Background

- Can we do better?
- Yes, if the bike types aren't equiprobable

Type, probability	# bits	code
$P(\text{Trek}) = 0.5$	1	1
$P(\text{Specialized}) = 0.25$	2	01
$P(\text{Cervelo}) = 0.125$	3	001
$P(\text{Serotta}) = 0.125$	3	000

- Optimal code uses $-\log_2 P(c)$ bits for event with probability $P(c)$

Information Theory Background

Type, probability	# bits	code
$P(\text{Trek}) = 0.5$	1	1
$P(\text{Specialized}) = 0.25$	2	01
$P(\text{Cervelo}) = 0.125$	3	001
$P(\text{Serotta}) = 0.125$	3	000

- Expected number of bits we have to communicate: 1.75 bits/bike

$$-\sum_{c=1}^{|C|} P(c) \log_2 P(c)$$

Entropy

- Entropy is a measure of uncertainty associated with a random variable

- Can be interpreted as the expected number of bits required to communicate the value of the variable

$$H(C) = -\sum_{c=1}^{|C|} P(c) \log_2 P(c)$$

entropy function for binary variable

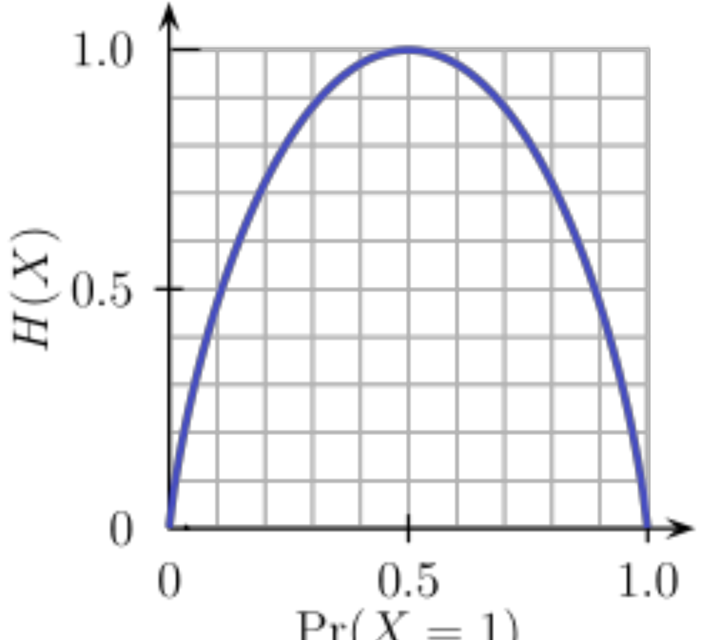


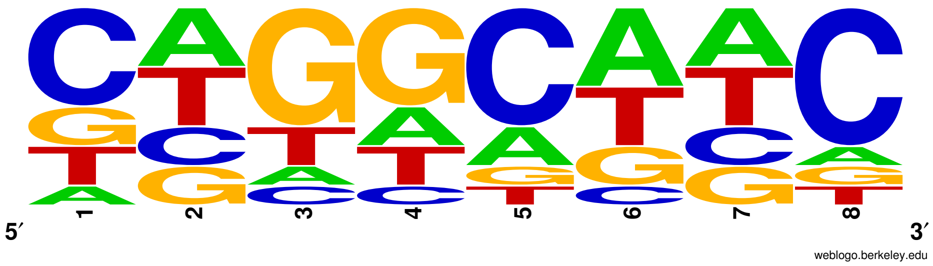
Image from Wikipedia

How is entropy related to DNA sequences?

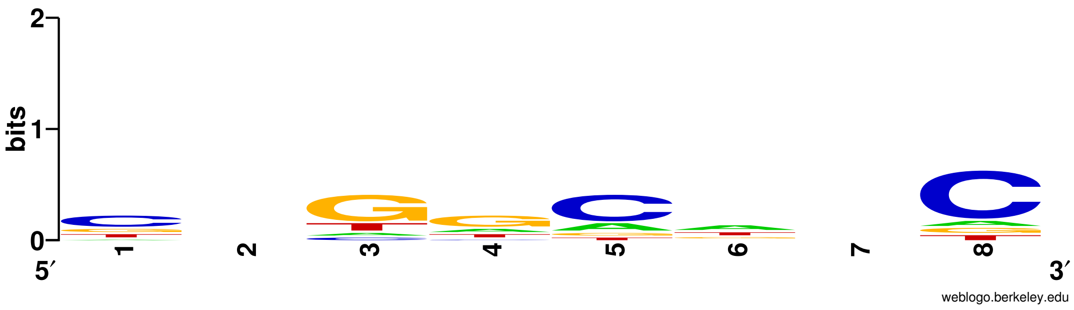
Sequence Logos

	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 ⇩

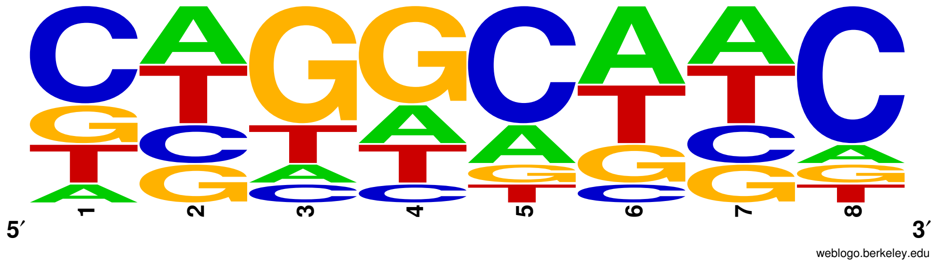


frequency logo



information content logo

Sequence Logos



- Typically represent a binding site

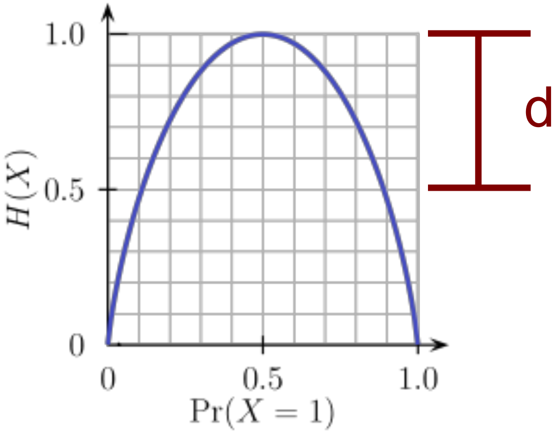
- Frequency logo: Height of each character c is proportional to $P(c)$
- Information content logo: based on entropy (*H*) of a random variable (*C*) representing distribution of character states at each position

Sequence Logos

- Height of logo at a given position determined by decrease in entropy (from maximum possible); i.e., information content

$$H_{\max} - H(C) = \log_2 N - \left(-\sum_c P(c) \log_2 P(c) \right)$$

\# of characters in alphabet



decrease in entropy

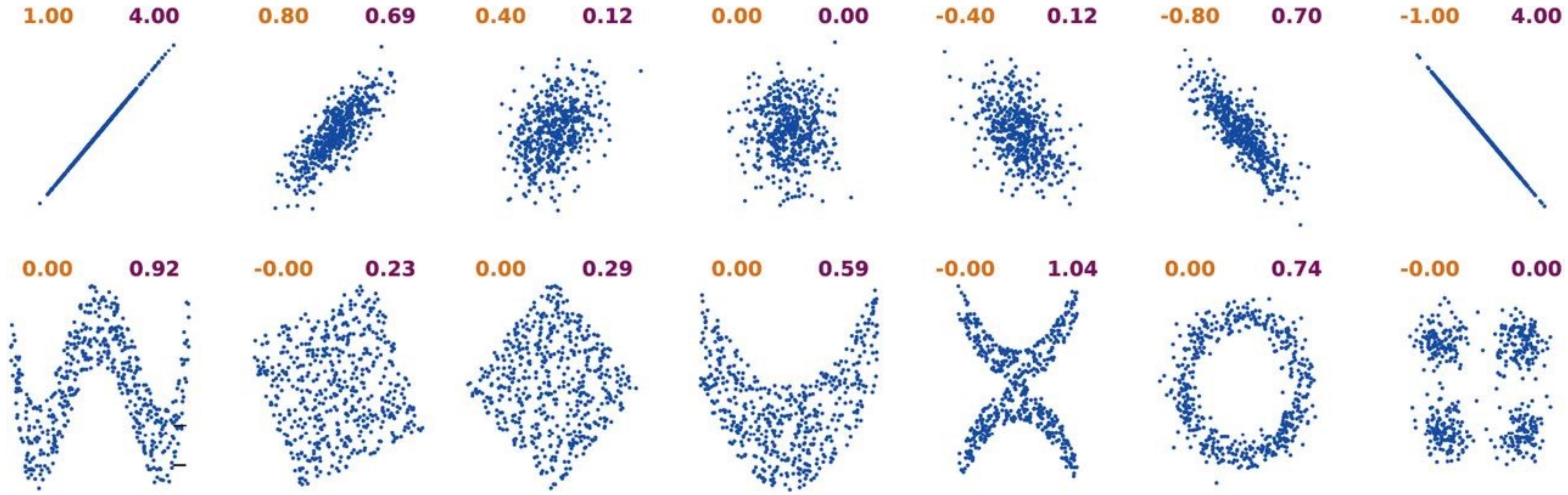
Mutual Information

- *Mutual information* quantifies how much knowing the value of one variable tells about the value of another

$$I(M;C) = \overset{\substack{\text{entropy of M}\\ \downarrow}}{H(M)} - \overset{\substack{\text{entropy of M}\\ \text{conditioned on C}\\ \swarrow}}{H(M \mid C)}$$

$$= \sum_{m} \sum_{c} P(m,c) \log_2 \left(\frac{P(m,c)}{P(m)P(c)} \right)$$

Correlation vs. Mutual information

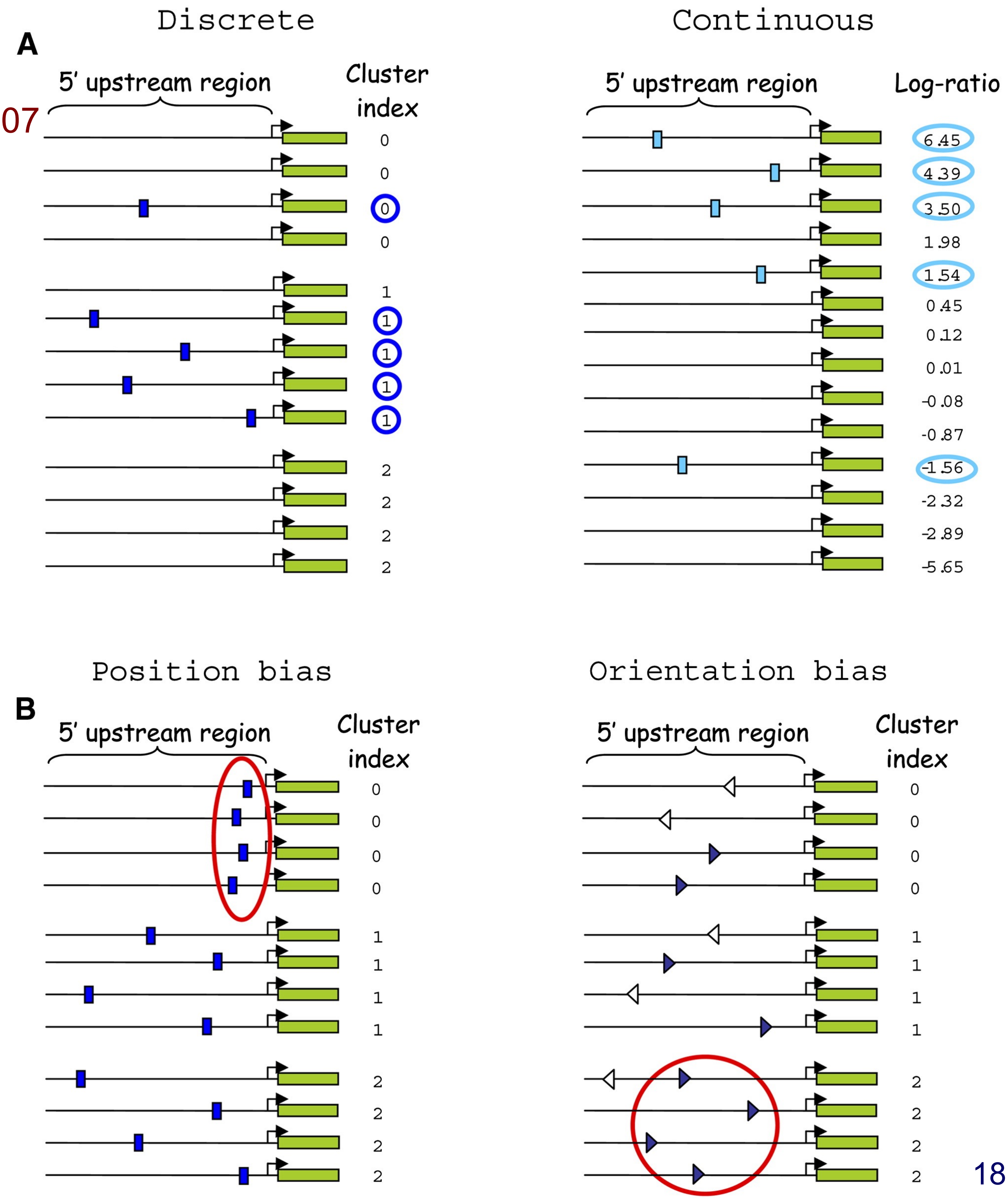


Human Brain Mapping, Volume: 38, Issue: 3, Pages: 1541-1573, First published: 17 November 2016, DOI: (10.1002/hbm.23471)

FIRE

Elemento et al., *Molecular Cell* 2007

- **F**inding **I**nformative **R**egulatory **E**lements (FIRE)
- **Given** a set of sequences grouped into clusters
- **Find** motifs, and relationships, that have high *mutual information* with the clusters
- Applicable when sequences have continuous values instead of cluster labels



Mutual Information in FIRE

- We can compute the mutual information between a motif and the clusters as follows

$$I(M;C) = \sum_{m=0}^{1} \sum_{c=1}^{|C|} P(m,c) \log_2 \frac{P(m,c)}{P(m)P(c)}$$

$m=0, 1$ represent absence/presence of motif

c ranges over the cluster labels

Finding Motifs in FIRE

- Motifs are represented by regular expressions; initially each motif is represented by a strict *k*-mer (e.g. TCCGTAC)

1. Test all *k*-mers (*k*=7 by default) to see which have significant mutual information with the cluster label
2. Filter *k*-mers using a significance test to obtain motif seeds
3. Generalize each motif seed
4. Filter motifs using a significance test

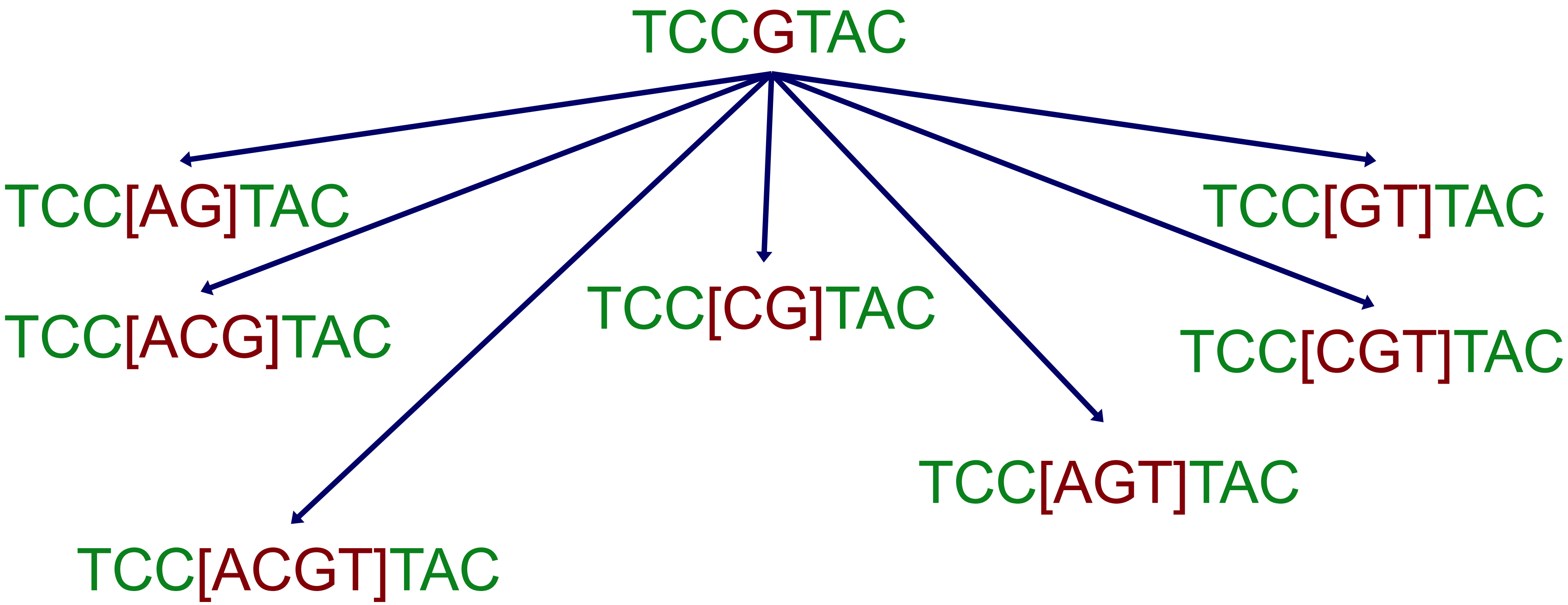
Significance test via randomization

- Given an empirical MI value for a motif, I
- Randomly shuffle cluster labels of genes (or other variables such as expression), and calculate MI
- Repeat shuffling N_r times and get N_r MI values
- Pseudo p-value = sum($I < N_r$ MI values)/N_r to see if it is less than a significance threshold (e.g., $1/N_r$)
  - Z-score = $(I - \mathrm{mean}(I_{\mathrm{random}}))/\mathrm{sigma}(I_{\mathrm{random}})$

Elemento et al., *Molecular Cell* 2007, Supplement

Key Step in Generalizing a Motif in FIRE

- Randomly pick a position in the motif
- Generalize in all ways consistent with current value at position
- Score each by computing mutual information
- Retain the best generalization



TCCGTAC

TCC[AG]TAC

TCC[GT]TAC

TCC[ACG]TAC

TCC[CG]TAC

TCC[CGT]TAC

TCC[AGT]TAC

TCC[ACGT]TAC

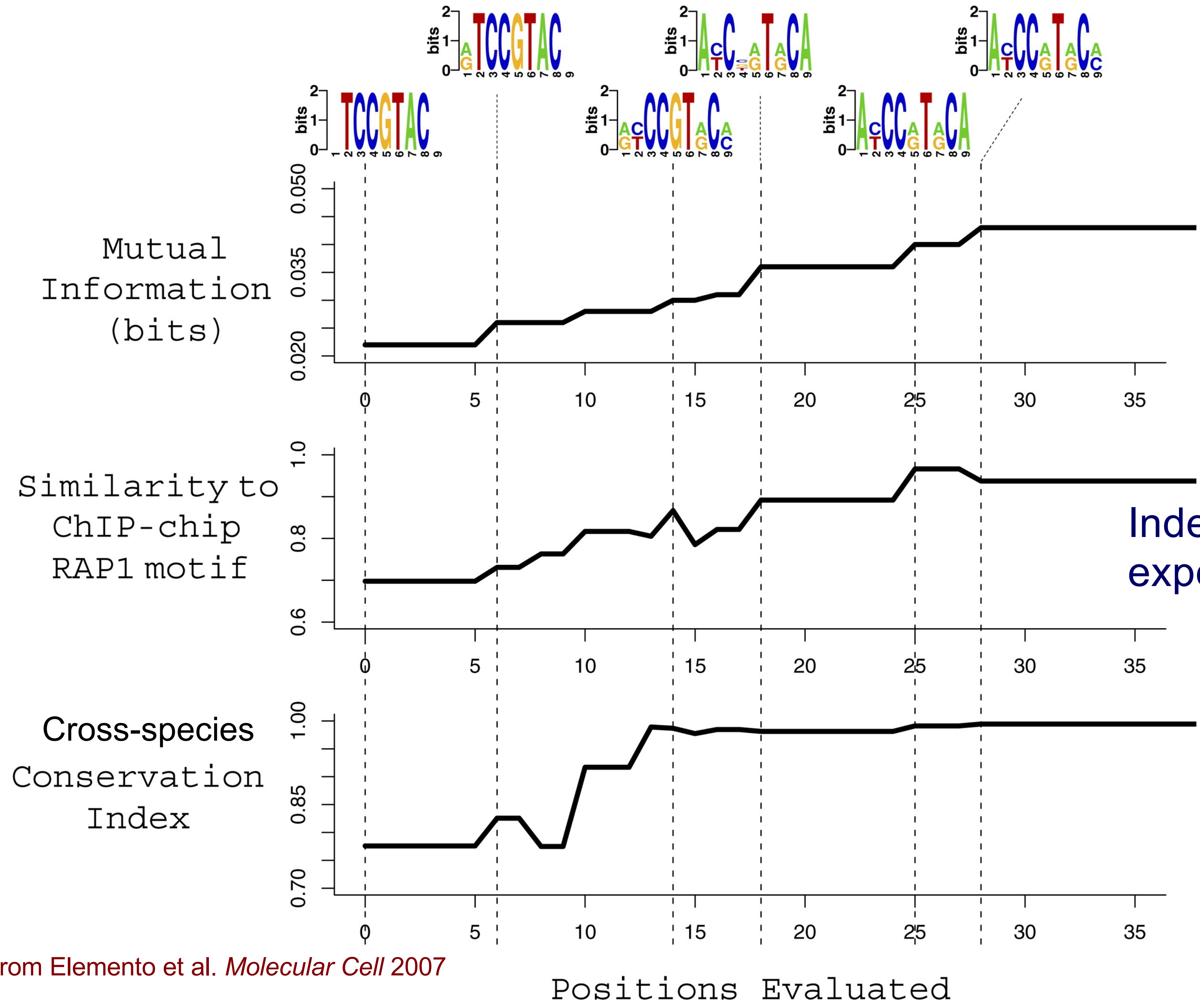
Generalizing a Motif in FIRE

```
given: k-mer, n

best ← null
repeat n times
    motif ← k-mer
    repeat
        motif ← GeneralizePosition(motif)    // shown on previous slide
    until convergence (no improvement at any position)
    if score(motif) > score(best)
        best ← motif

return: best
```

Generalizing a Motif in FIRE: Example



Independent experiment

Figure from Elemento et al. *Molecular Cell* 2007

Avoiding Redundant Motifs

- Different seeds could converge to similar motifs

TCCGTAC → TCC[CG]TAC

TCCCTAC → TCC[CG]TAC

- Use mutual information to test whether new motif is unique and contributes new information

$$\frac{I(M;C\mid M')}{I(M;M')} > r$$

M' previous motif

M new candidate motif

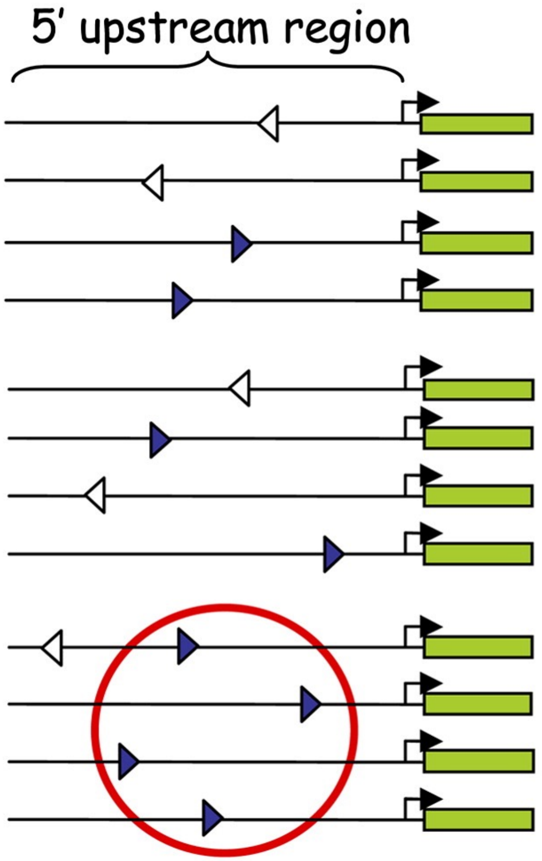
C expression clusters

Characterizing Predicted Motifs in FIRE

- Mutual information is also used to assess various properties of found motifs
  - orientation bias
  - position bias
  - interaction with another motif

Using MI to Determine Orientation Bias

$I(S;C)$ C indicates cluster
$S=1$ indicates motif present on transcribed strand
$S=0$ otherwise (not present or not on transcribed strand)



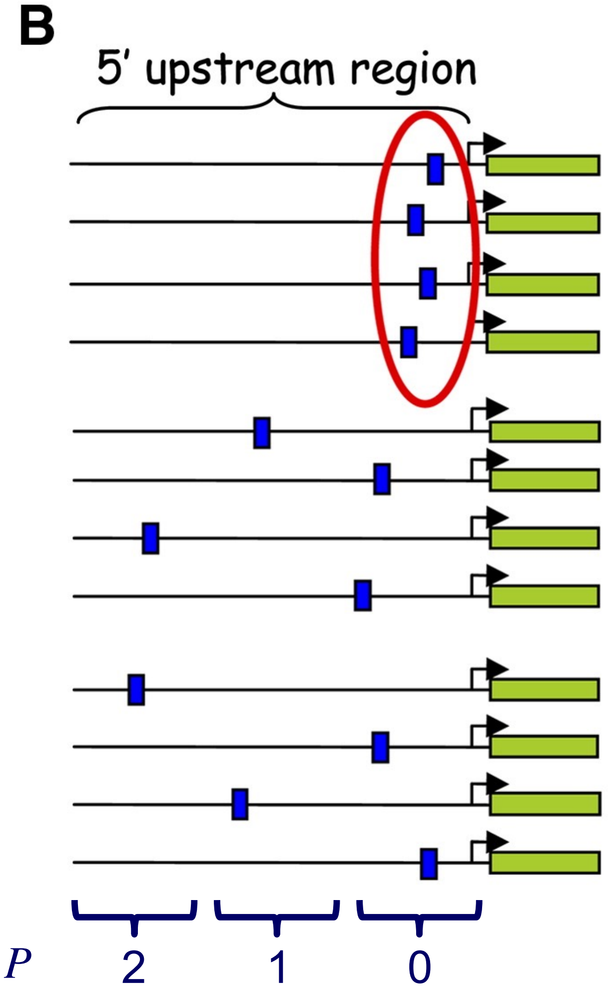
C	S
0	0
0	0
0	1
0	1
1	0
1	1
1	0
1	1
2	1
2	1
2	1
2	1

Also compute MI where S=1 indicates motif present on complementary strand

Using MI to Determine Position Bias

$I(P;O)$ P ranges over position bins

O=0, 1 indicates whether or not the motif is over-represented in a sequence's cluster

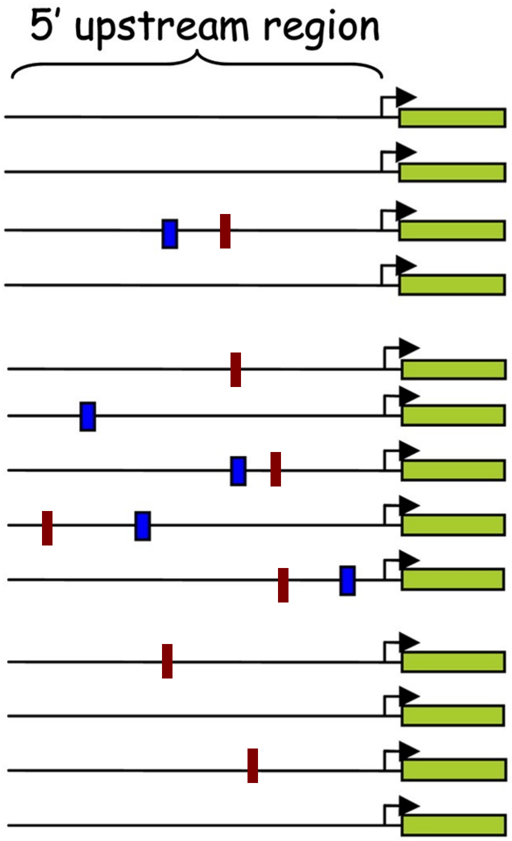


P	O
0	1
0	1
0	1
0	1
1	0
0	0
2	0
0	0
2	0
0	0
1	0
0	0

Only sequences containing the motif are considered for this calculation

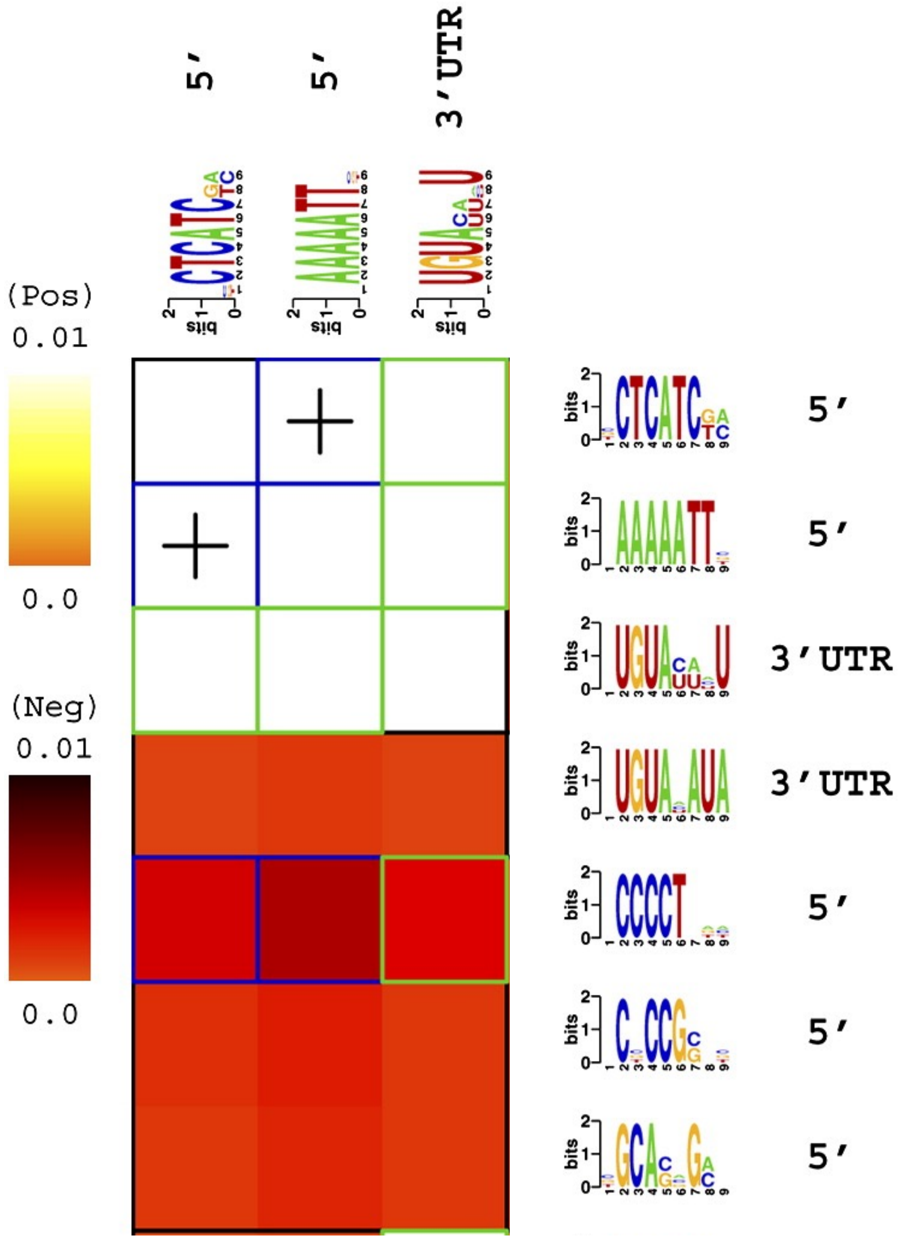
Using MI to Determine Motif Interactions

$I(M_1; M_2)$ $M_1=0, 1$ indicates whether or not a sequence has the motif **and** is in a cluster for which the motif is over-represented; similarly for M_2



M_1	M_2
0	0
0	0
0	0
0	0
0	1
1	0
1	1
1	1
1	1
0	1
0	0
0	1
0	0

Motif Interactions Example



Yeast motif-motif interactions

White: positive association

Dark red: negative association

Blue box: DNA-DNA

Green box: DNA-RNA

Plus: spatial co-localization

Discussion of FIRE

- FIRE
  - mutual information used to identify motifs and relationships among them
  - motif search is based on generalizing informative *k*-mers

- Consider advantages and disadvantages of *k*-mers versus PWMs

- In contrast to many motif-finding approaches, FIRE takes advantage of *negative* sequences

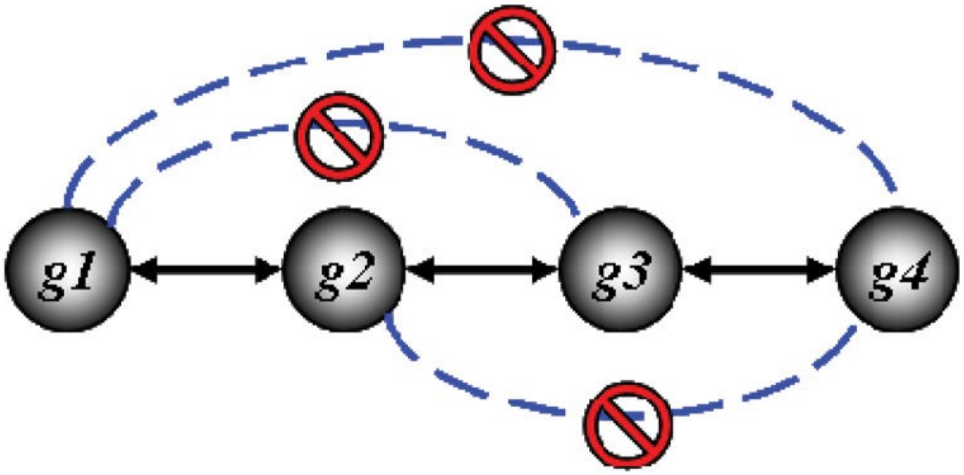
- FIRE returns all informative motifs found

Mutual Information for Gene Networks

- Mutual information and conditional mutual information can also be useful for reconstructing biological networks
- Build gene-gene network where edges indicate high MI in genes’ expression levels
- Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNE)

ARACNE

- Gaussian kernel estimator to estimate mutual information
  - No binning or histograms
- Data processing inequality
  - Prune indirect edges



Margolin et al. *BMC Bioinformatics* 2006