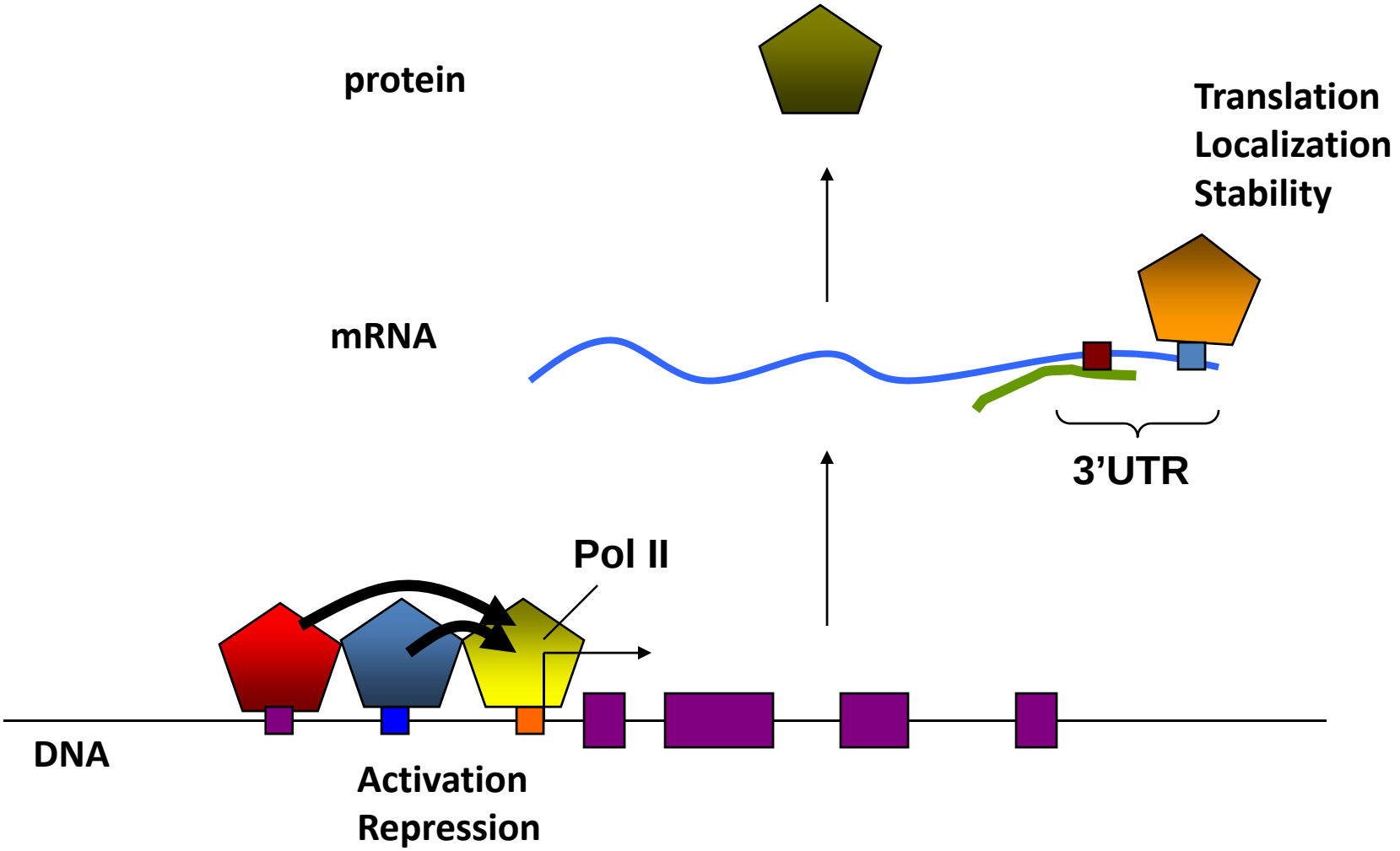
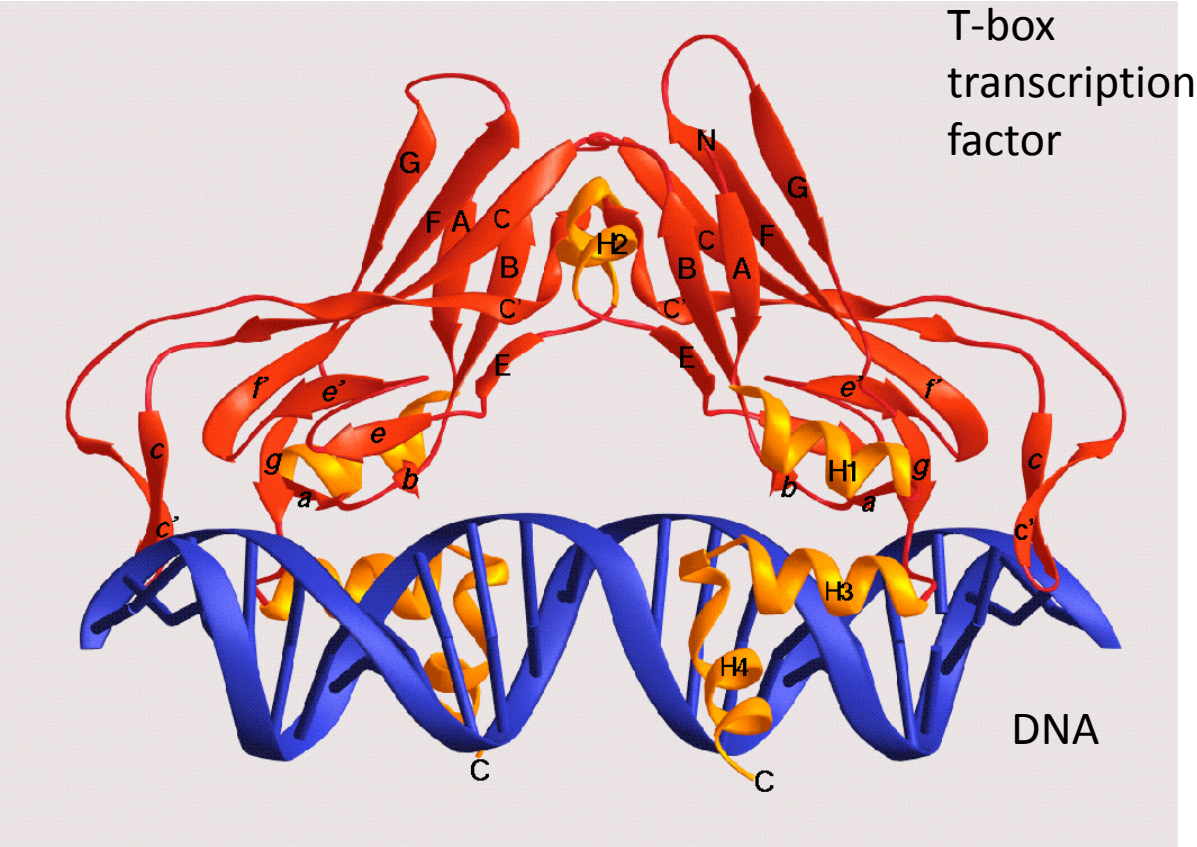
Discovering regulatory sequences from expression data

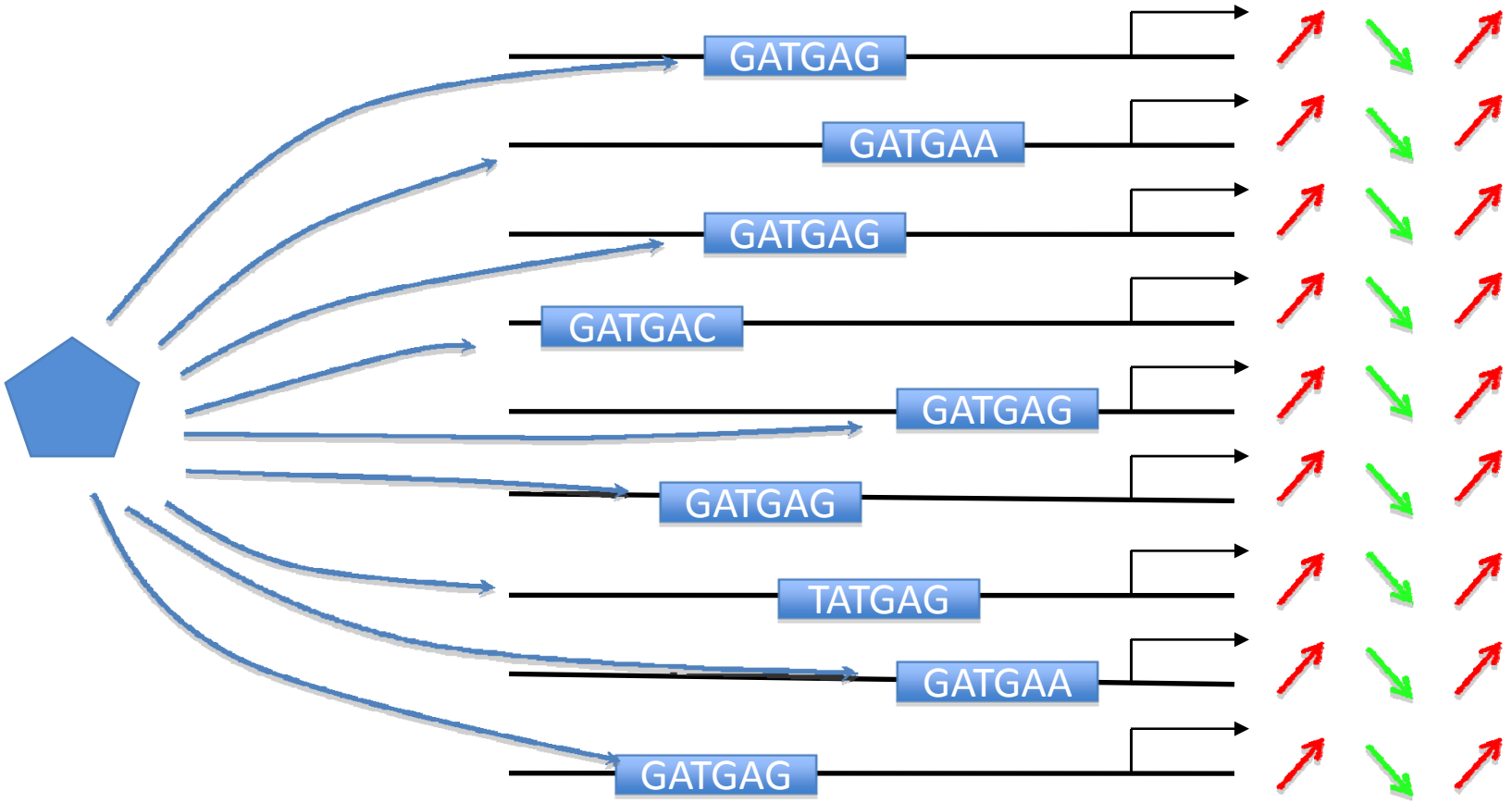
- Unsupervised clustering
- Information theory
- Optimization
- Non-parametric statistical testing
- Multiple testing
- Overfitting

Transcriptional and post-transcriptional regulation of gene expression

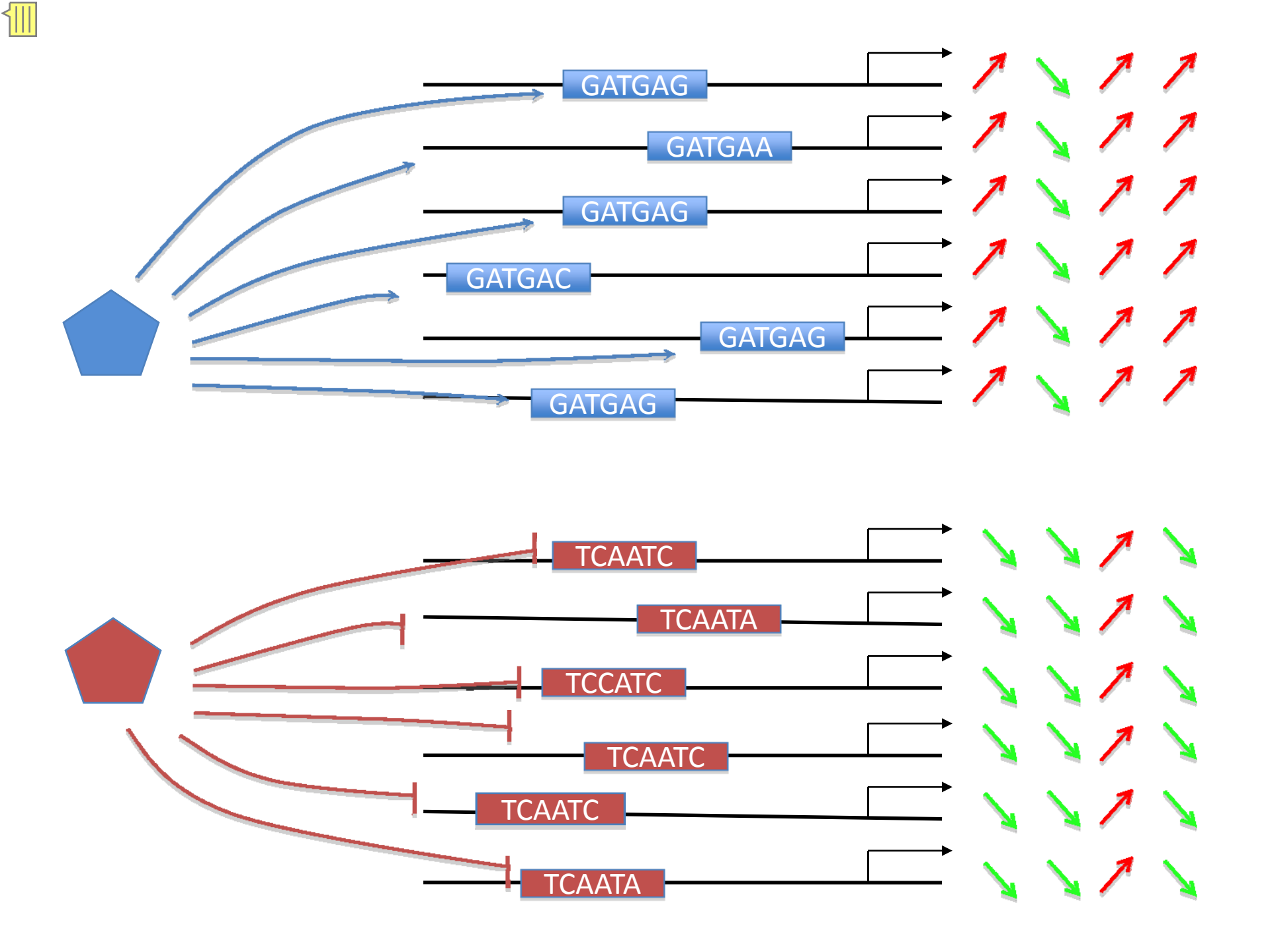


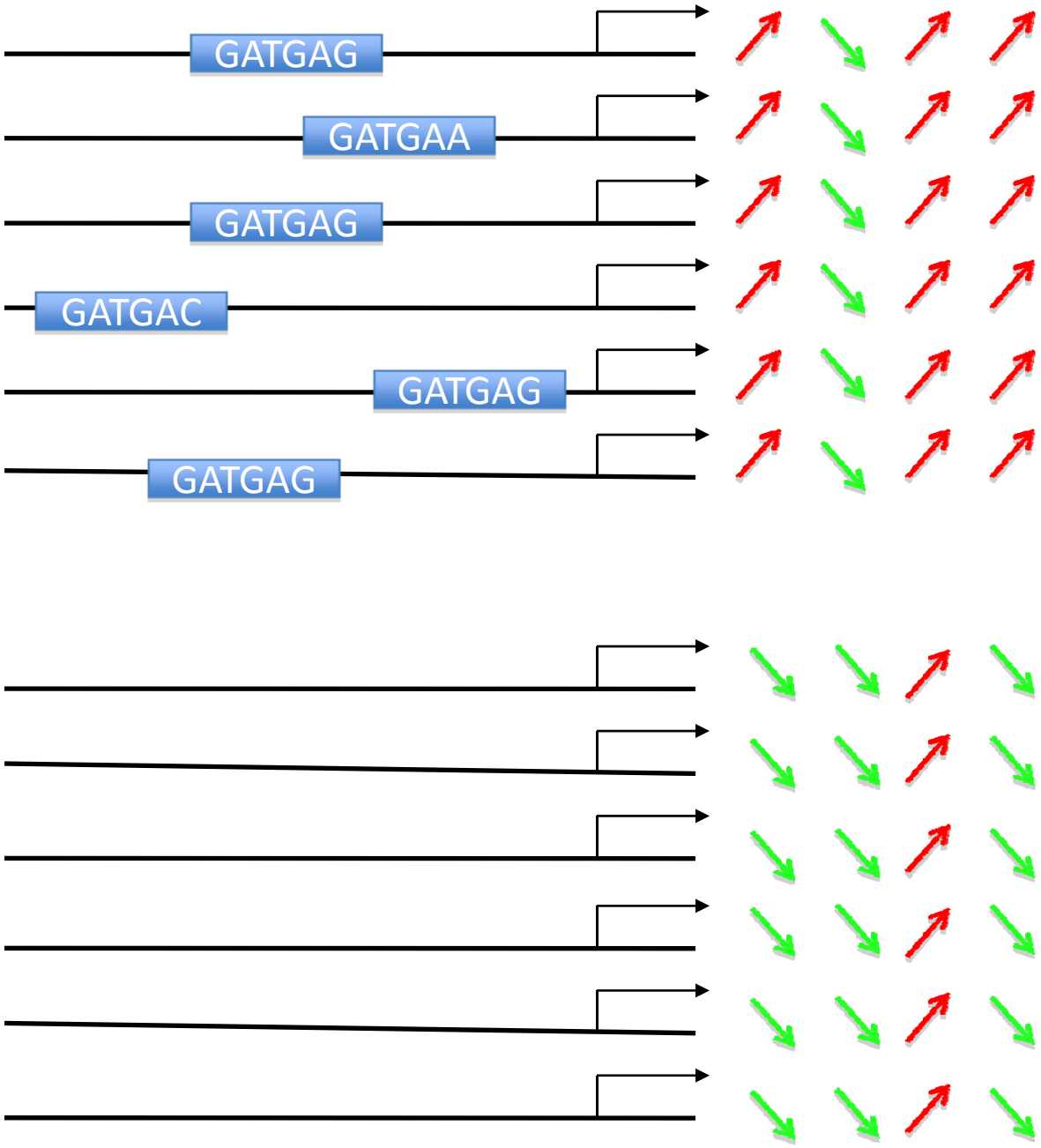


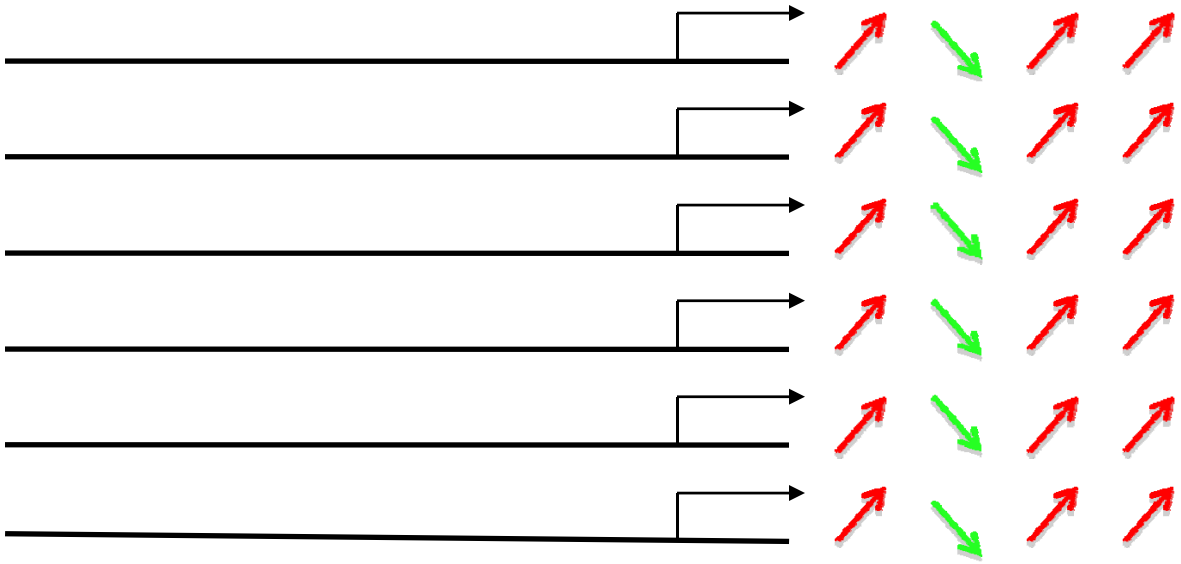
Transcription factor binding sites are ~6-12 bp-long

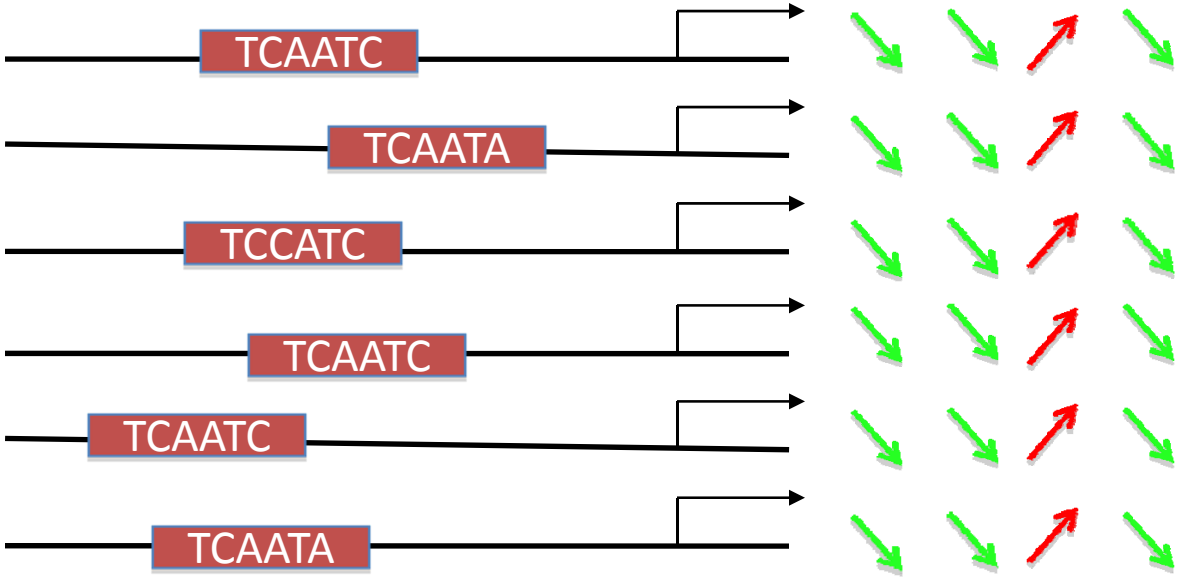


Genes regulated by the same TF will be co-expressed


GATGAG
GATGAA
GATGAG
GATGAC
GATGAG
GATGAG
TCAATC
TCAATA
TCCATC
TCAATC
TCAATC
TCAATA

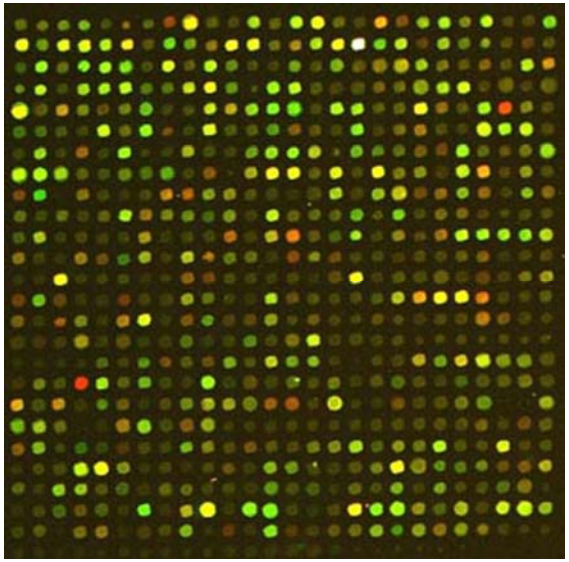

GATGAG
GATGAA
GATGAG
GATGAC
GATGAG
GATGAG

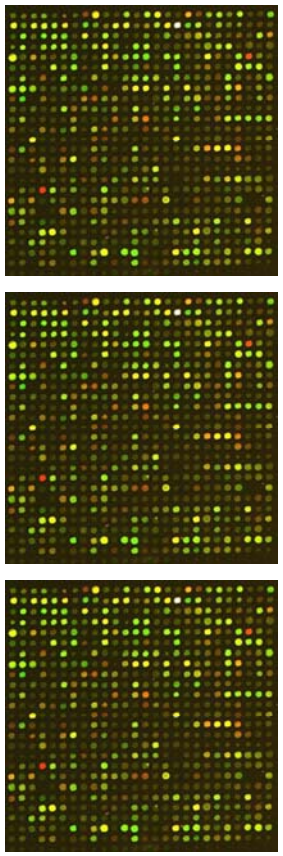



TCAATC
TCAATA
TCCATC
TCAATC
TCAATC
TCAATA

Several microarray experiments (conditions, time points, treatments)

Microarray experiment





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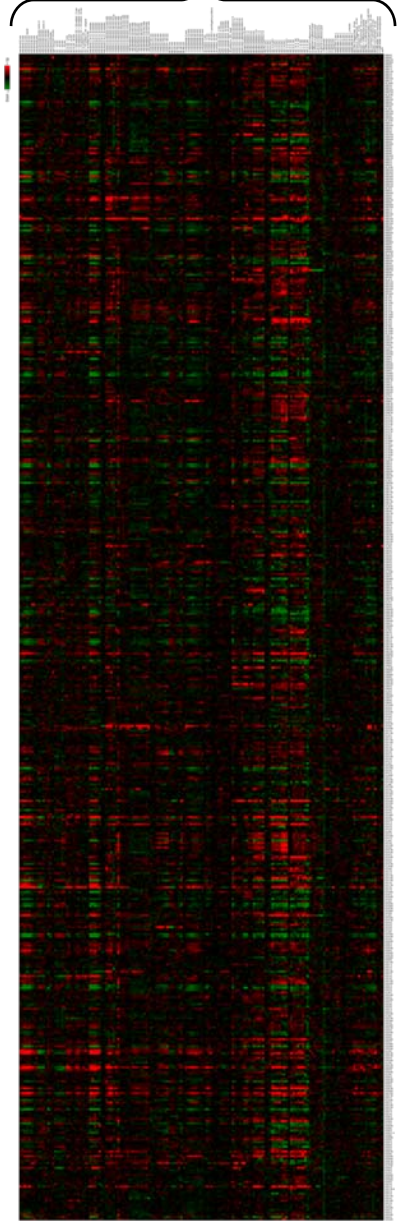
Microarray Experiments

Genes

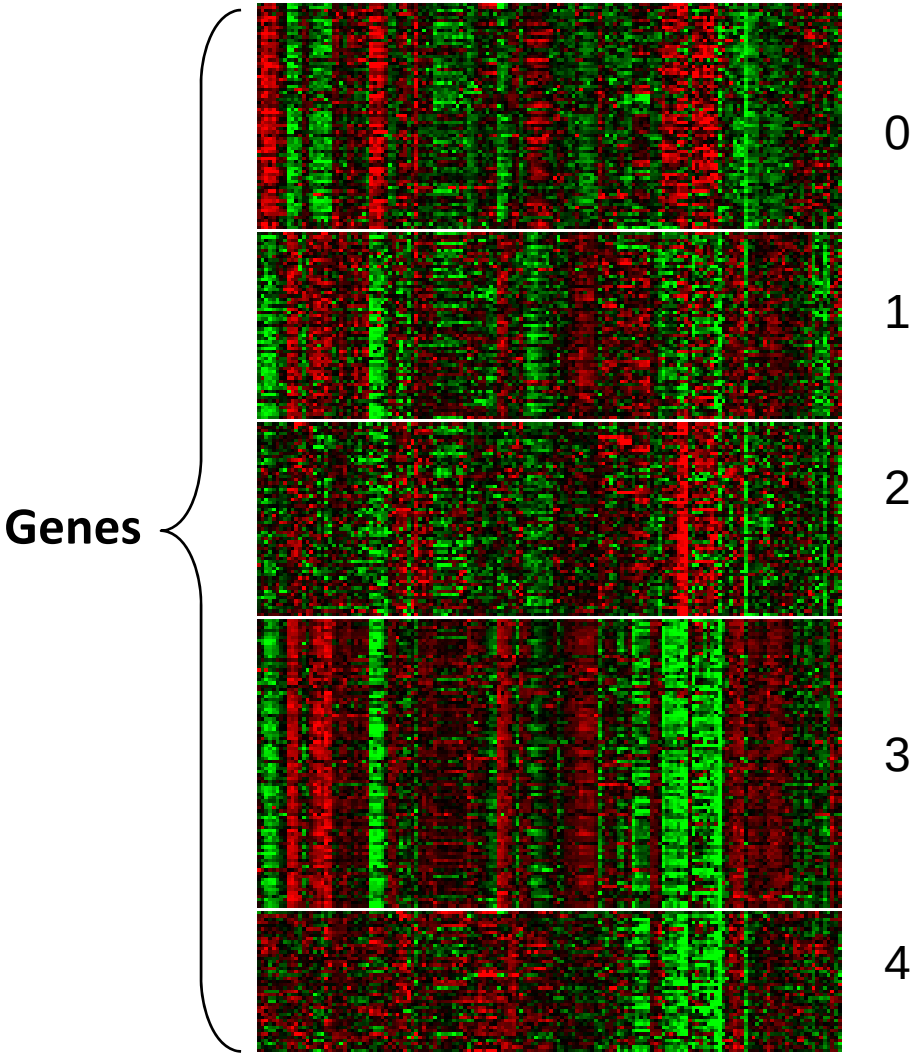


Creating co-expression clusters

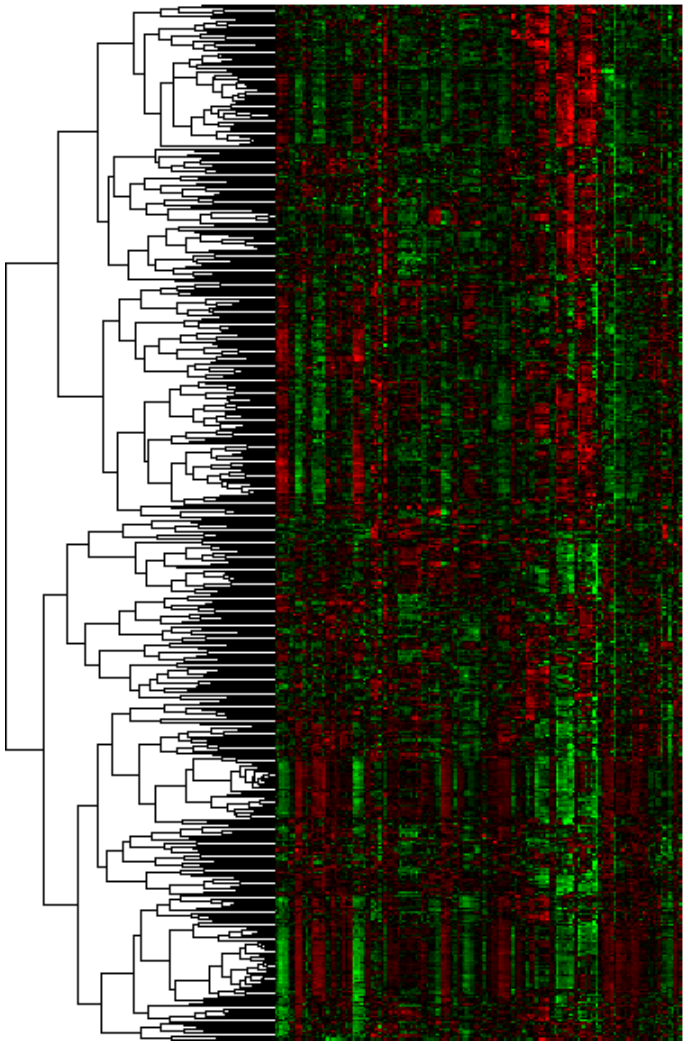
Unsupervised clustering approaches:

- K-means
- Self-organizing maps
- Hierarchical clustering

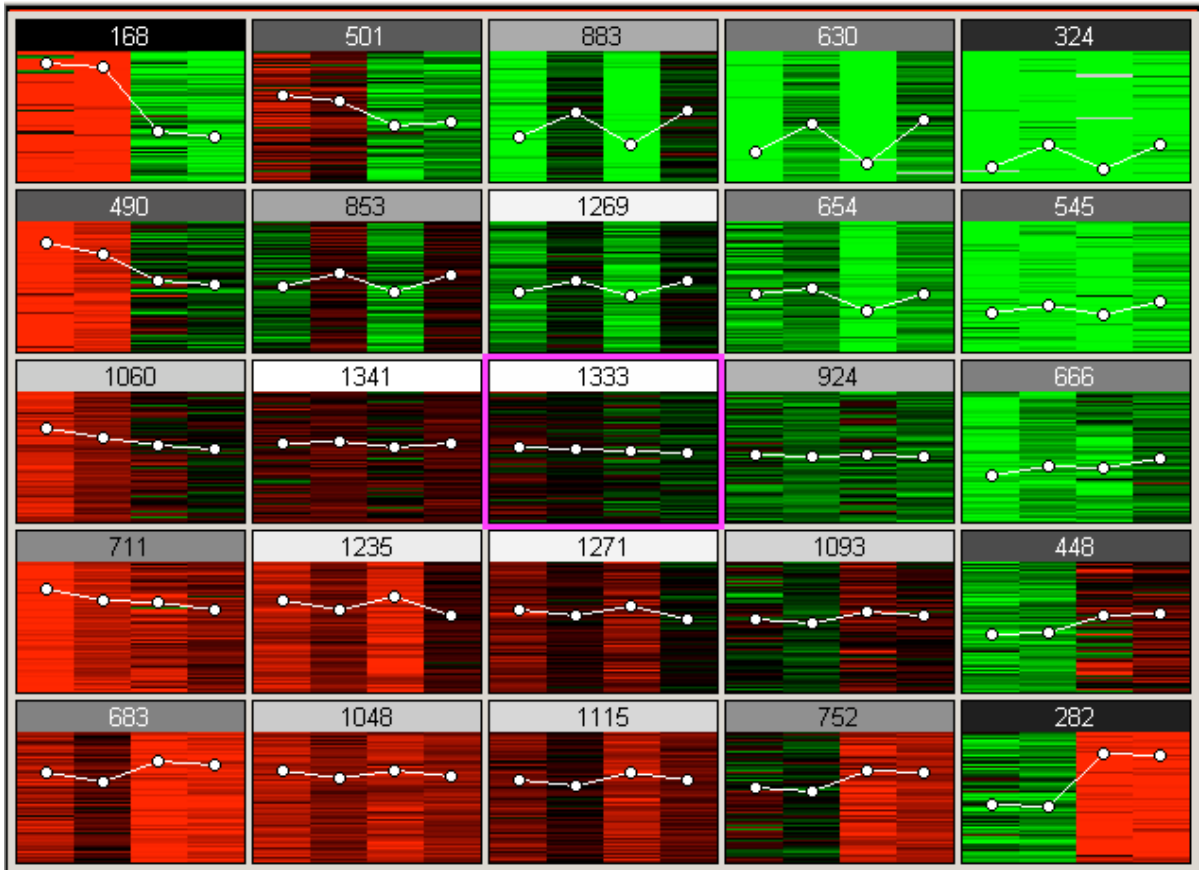
K-means clustering



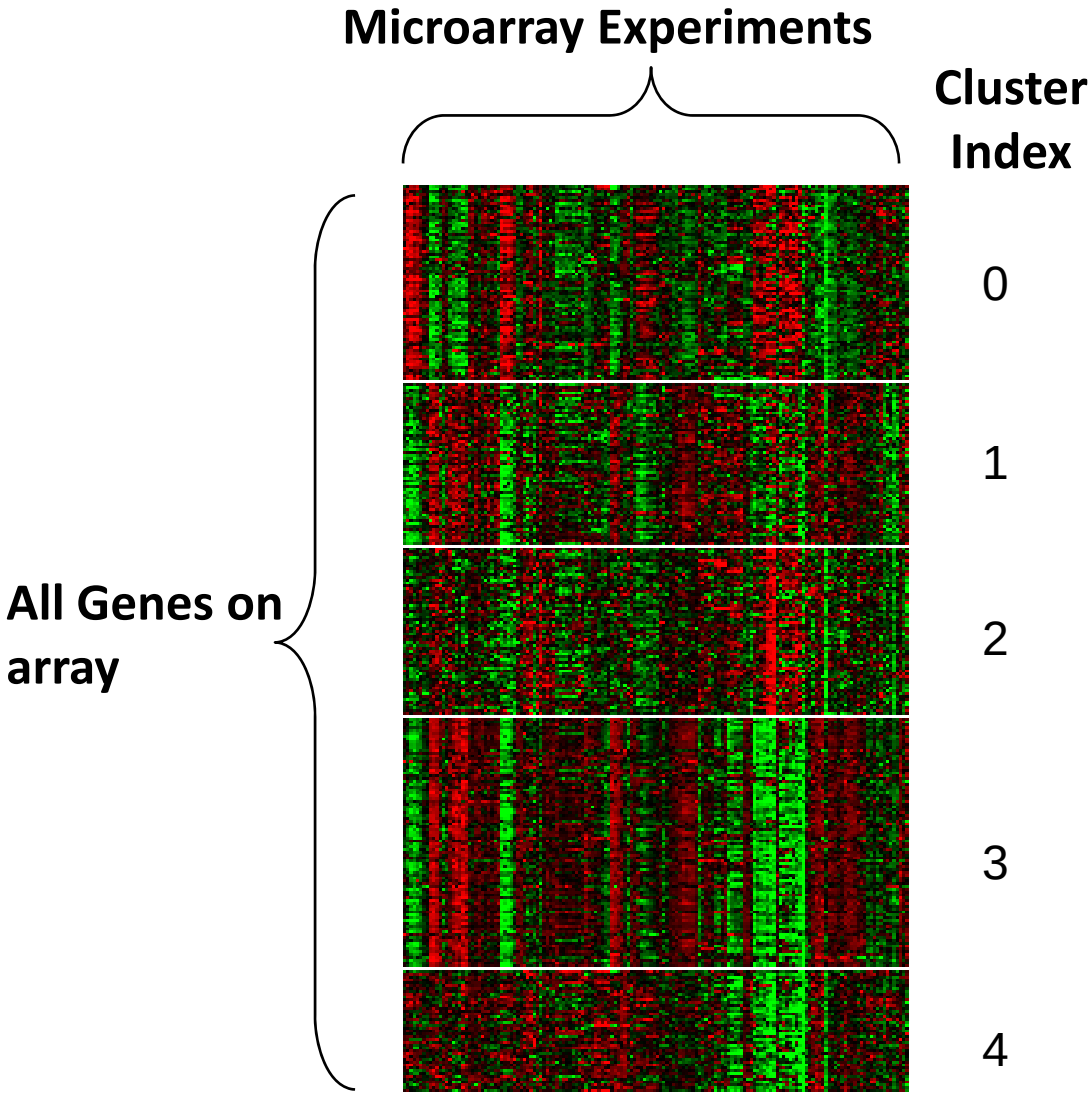
Hierarchical clustering

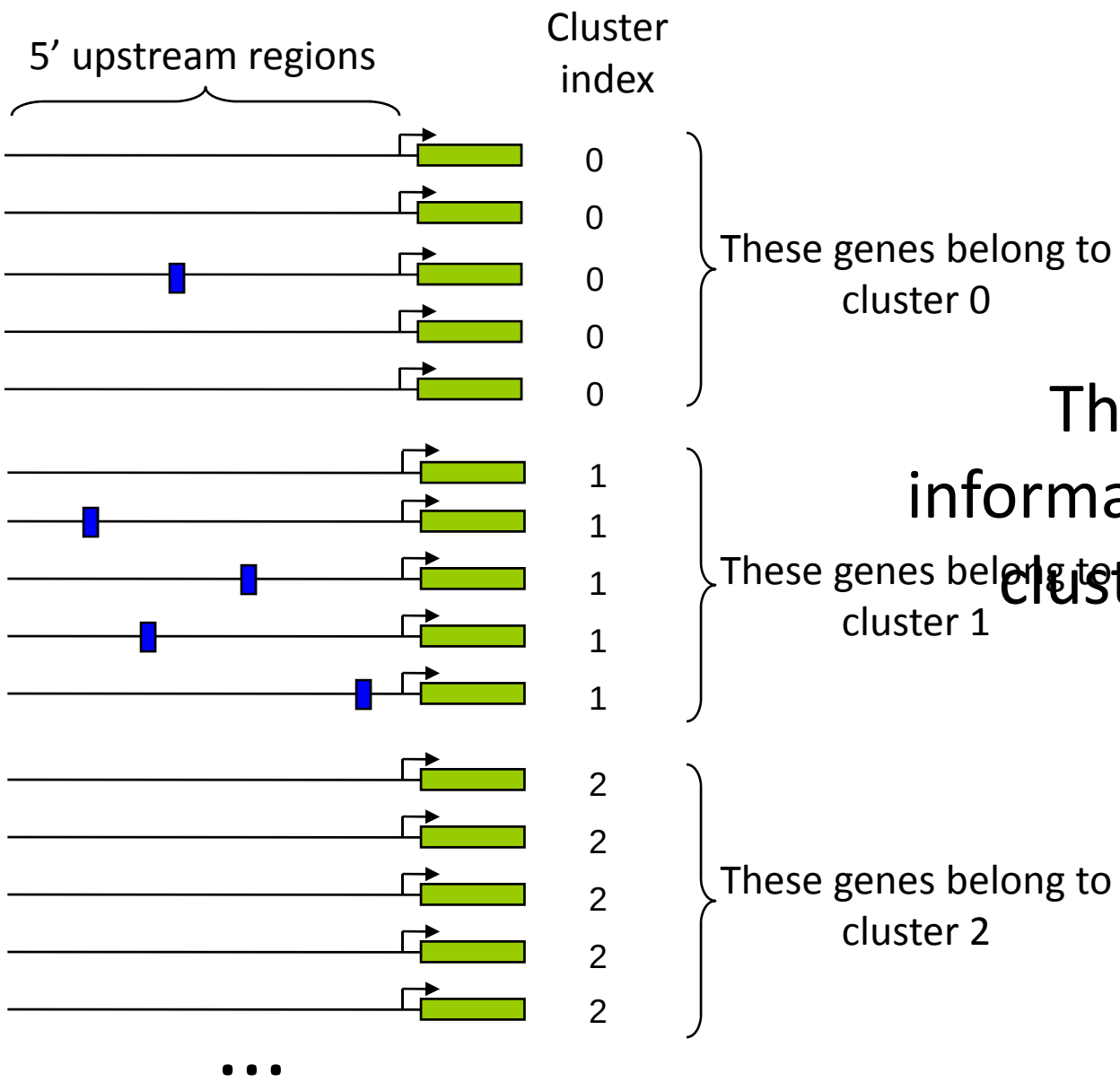


Self-organizing map



Clusters of co-expressed genes





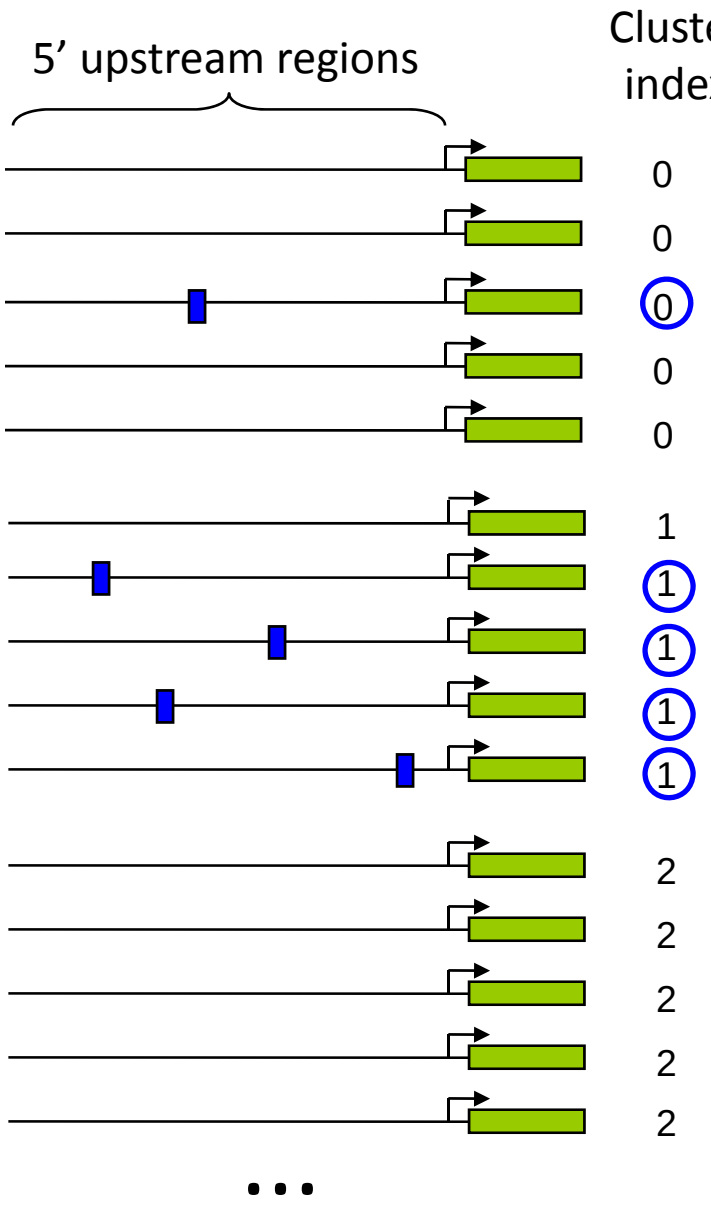
This motif is informative about the cluster indices !

Mutual Information

$$I(\mathrm{X};\mathrm{Y}) = \sum_{x}\sum_{y} P(x,y)\log\frac{P(x,y)}{P(x)P(y)}$$

I(X;Y) quantifies the amount of information that a variable X contains about another variable Y

Expressed in bits



Expression (Cluster Indices)

Motif	0	1	2
Absent	0.27	0.07	0.33
Present	0.07	0.27	0.00

$$I(\text{motif};\text{expression}) = \sum_{i=1}^{2}\sum_{j=1}^{3} P(i,j)\log\frac{P(i,j)}{P(i)P(j)}$$

I(M;E) = 0.34 bits

MI estimator is biased (sample size bias)

N=10

X1={1,0,0,1,0,1,1,0,1,0}

X2={0,0,1,1,0,1,0,1,1,0}

I(X1;X2) =?

100,000 times

N=100

X1={1,0,0,1,…. ,1,0,1,0}

X2={0,0,1,1,…. ,0,1,1,0}

I(X1;X2) =?

100,000 times

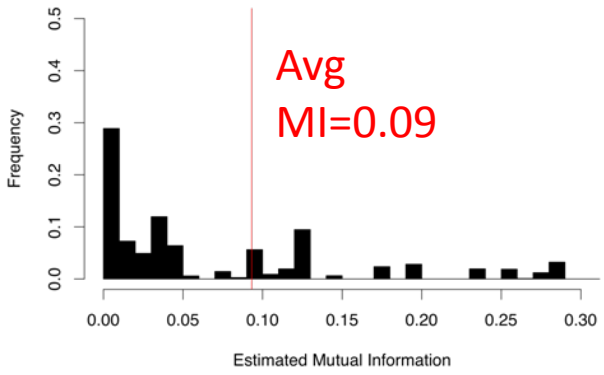
N=1000

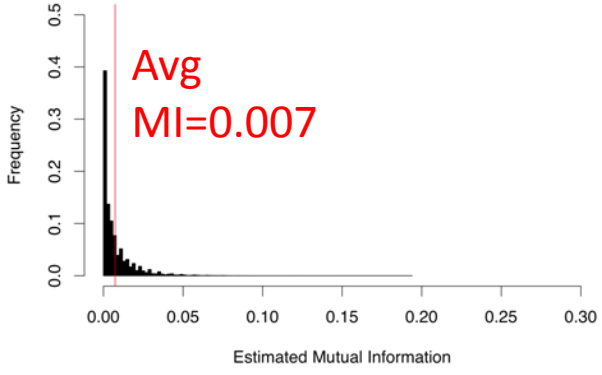
X1={1,0,0,1,…. ,1,0,1,0}

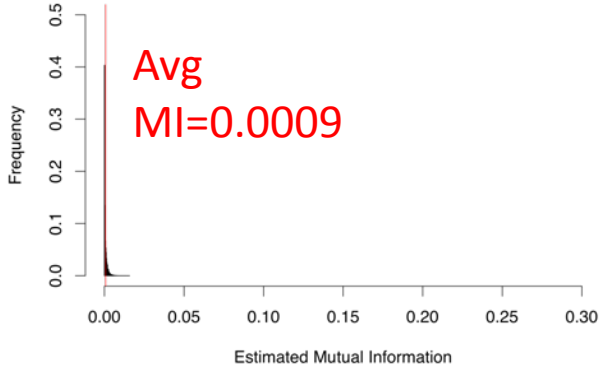
X2={0,0,1,1,…. ,0,1,1,0}

I(X1;X2) =?

100,000 times







MI estimator is biased (sample size bias)

- Can correct for sample size bias, e.g. Slonim et al, 2002 … slow … not very precise … not necessary if:
- Keep sample size the same so that we can compare MI values
- Estimate how large an MI value is compared to expectated MI

Algorithm for finding informative motifs

motif representations

	Accuracy	Search space
Degenerate code [AC]CGATGAG[TC]	good	large
Words (*k*-mers) GCGATGAG	acceptable	small

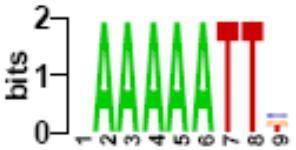
Motif Search Algorithm

Highly informative

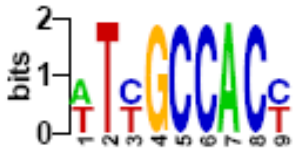
k-mer	MI
CTCATCG	0.0618
TCATCGC	0.0485
AAAATTT	0.0438
GATGAGC	0.0434
AAAAATT	0.0383
ATGAGCT	0.0334
TTGCCAC	0.0322
TGCCACC	0.0298
ATCTCAT	0.0265
...	
...	
ACGCGCG	0.0018
CGACGCG	0.0012
TACGCTA	0.0011
ACCCCCT	0.0010
CCACGGC	0.0009
TTCAAAA	0.0005
AGACGCG	0.0004
CGAGAGC	0.0003
CTTATTA	0.0002

Not informative

MI=0.081



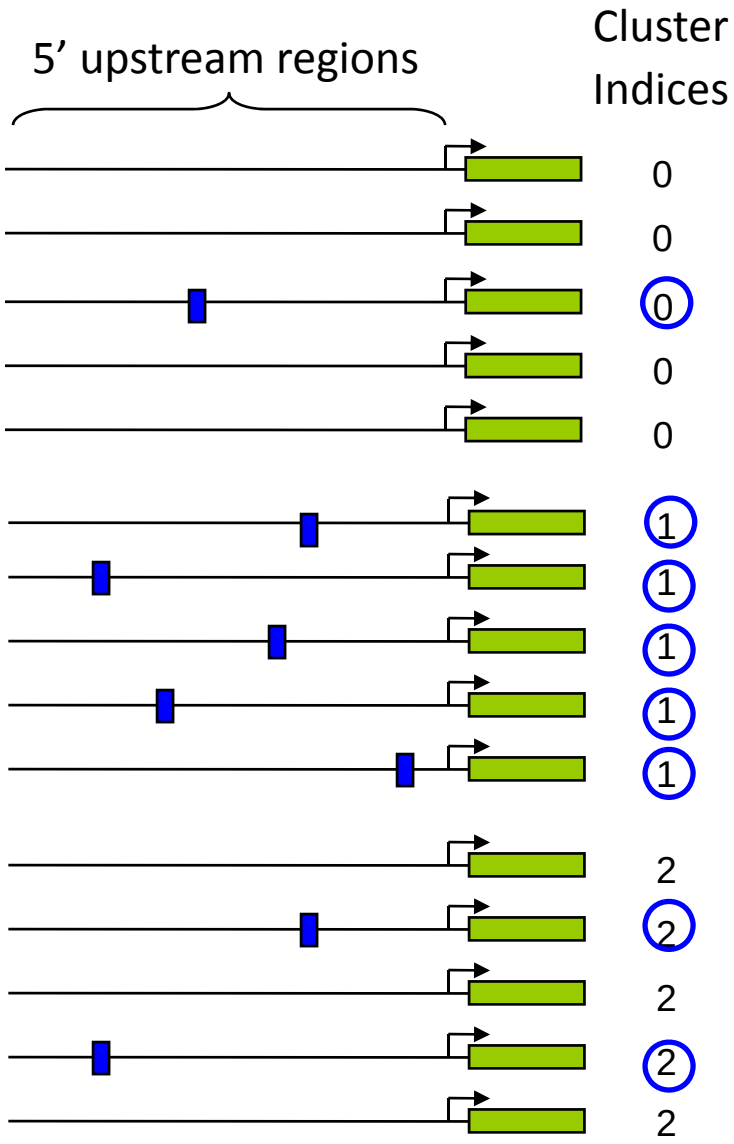
MI=0.045

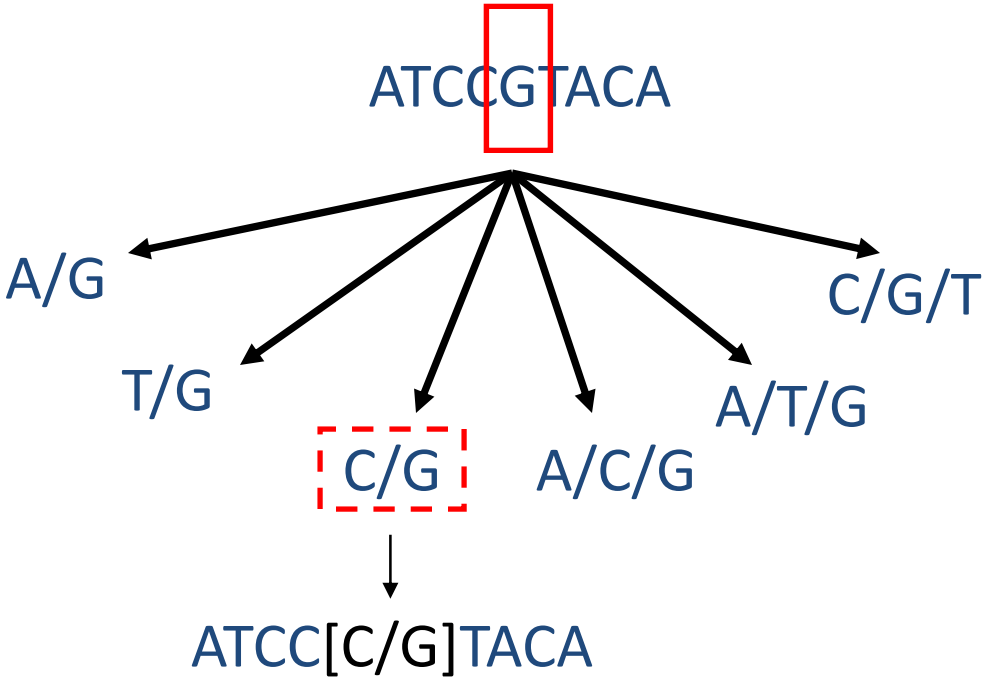


MI=0.040

...

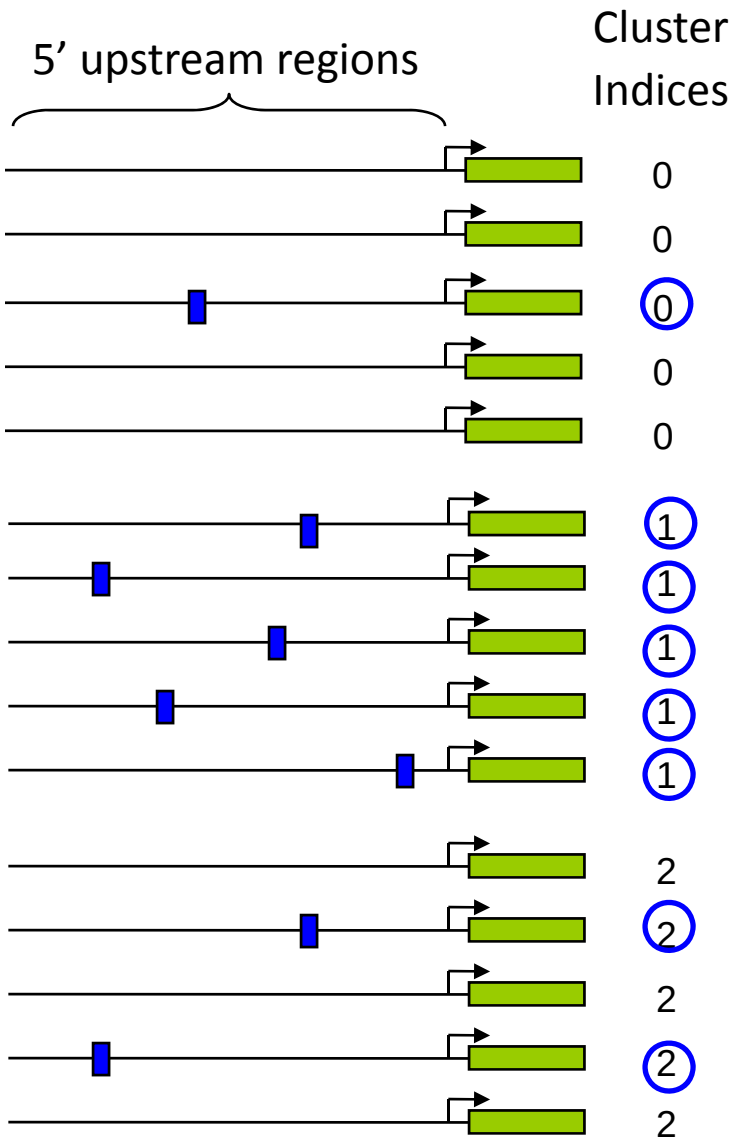
Optimizing *k*-mers into more informative degenerate motifs

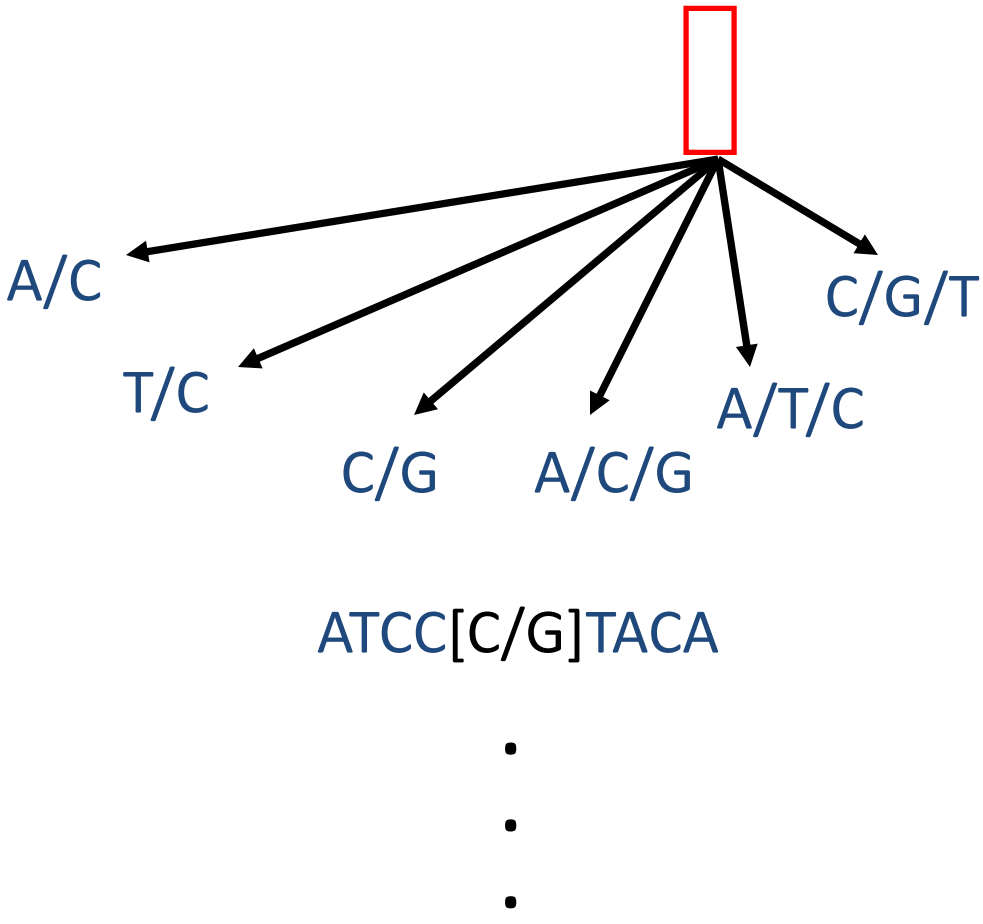




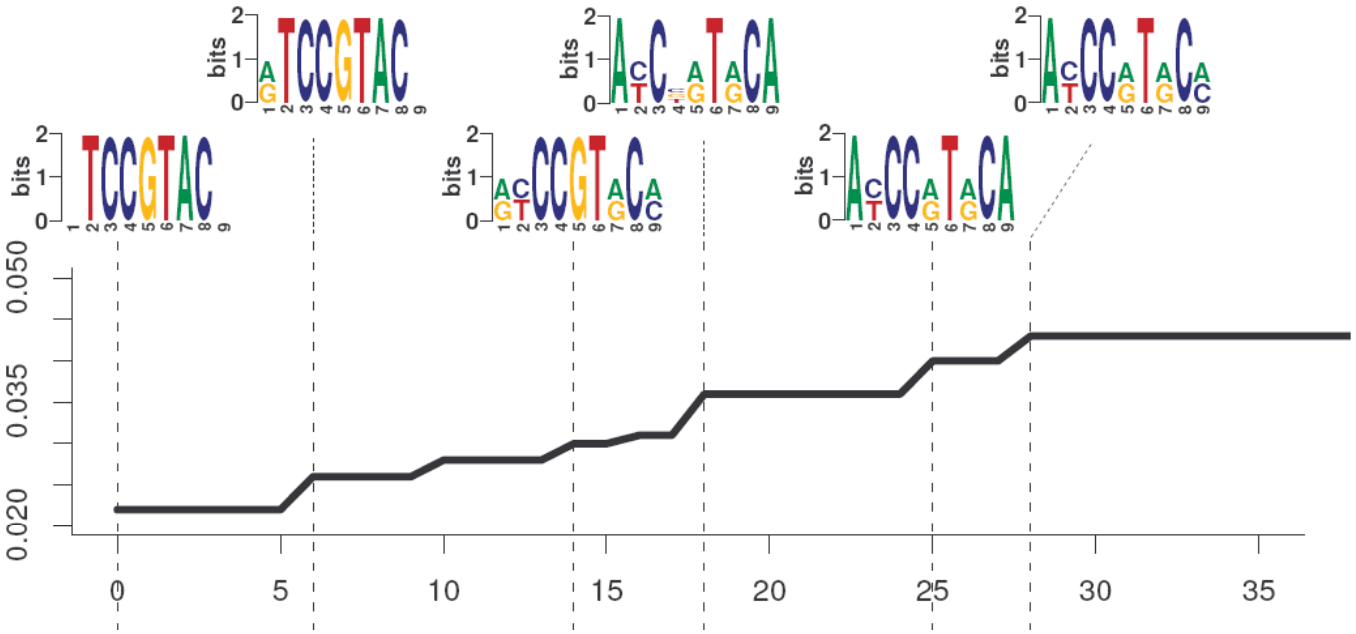
which character increases the mutual information by the largest amount ?

Optimizing *k*-mers into more informative degenerate motifs



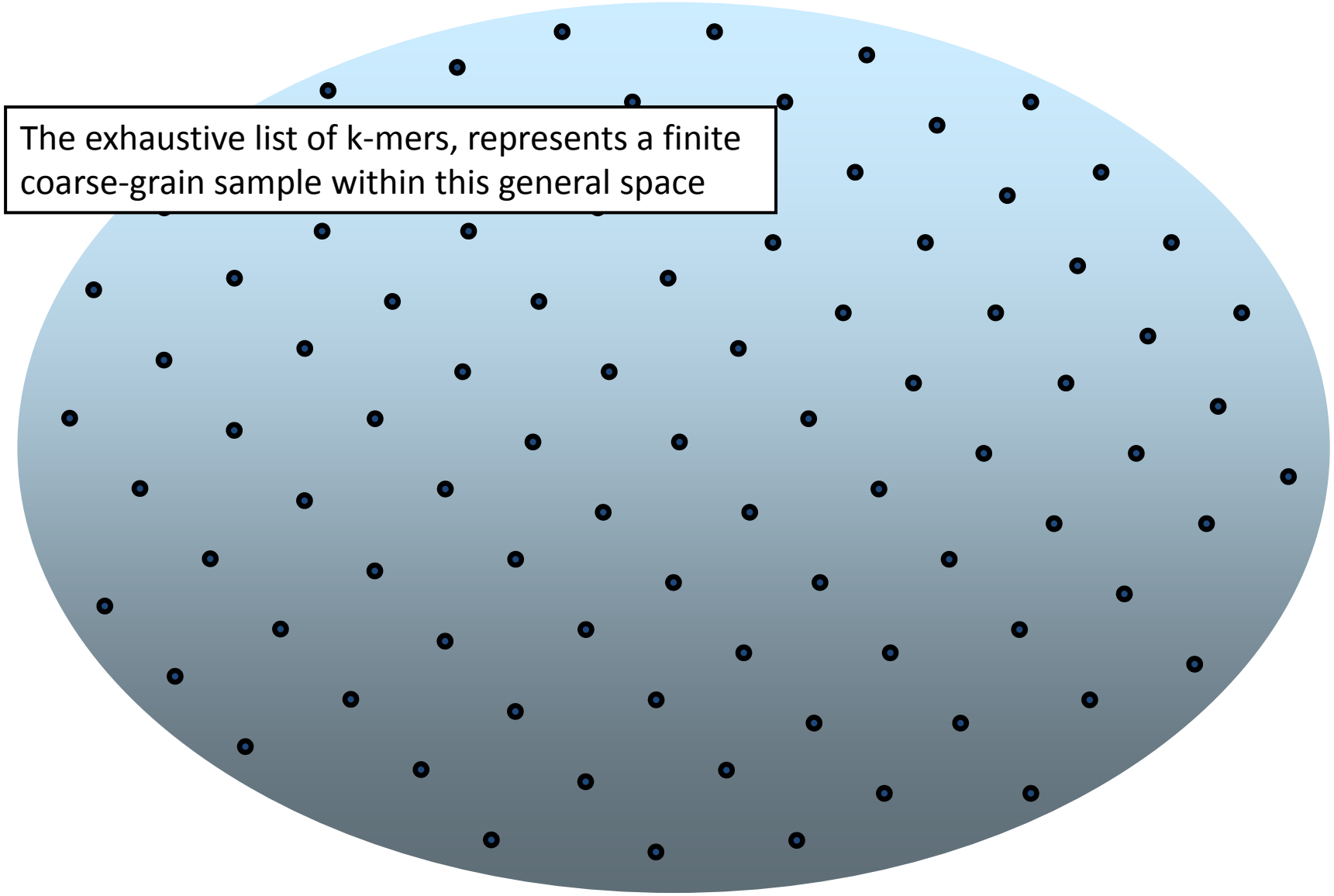


Mutual information

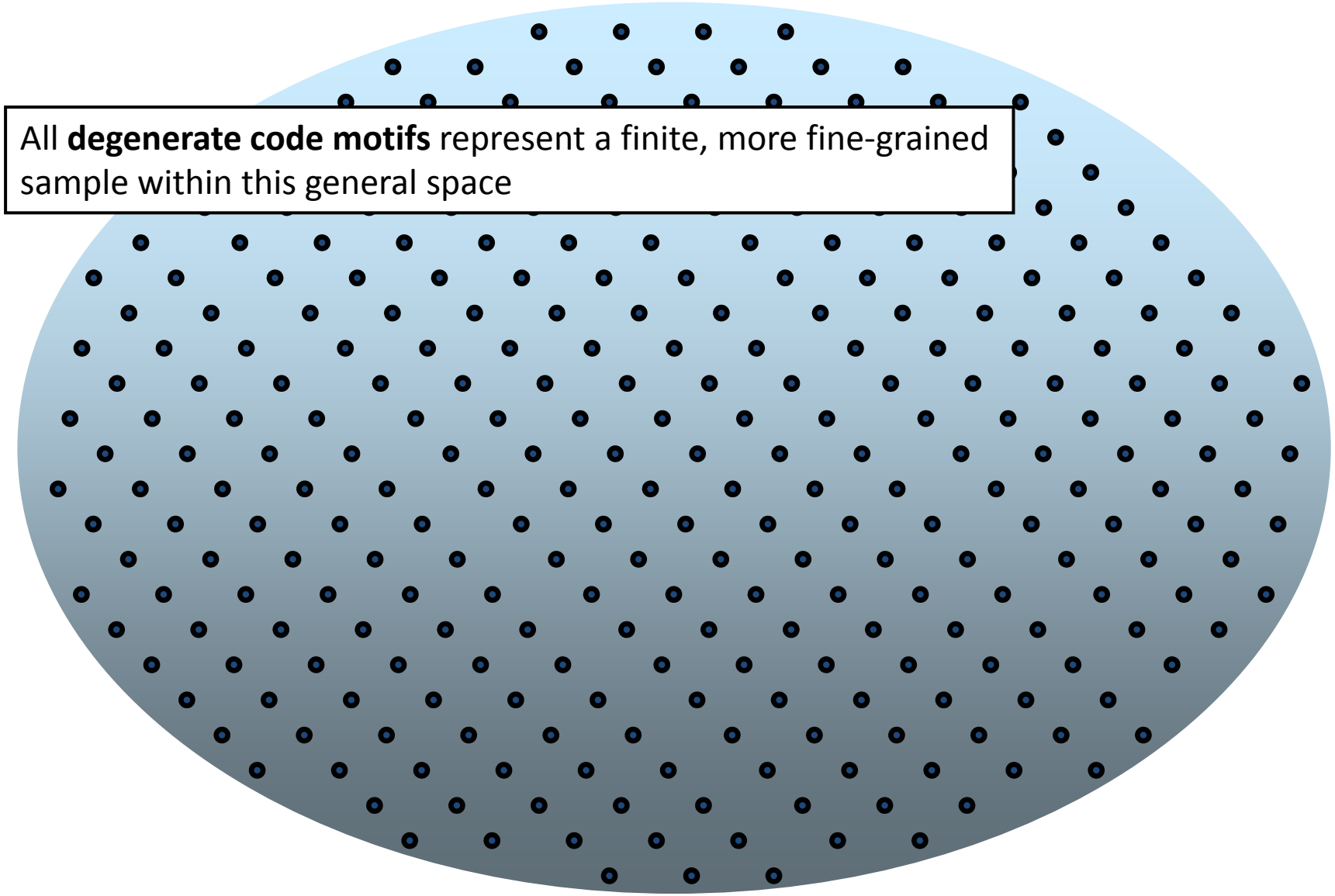


Optimization iterations (visited motif positions)

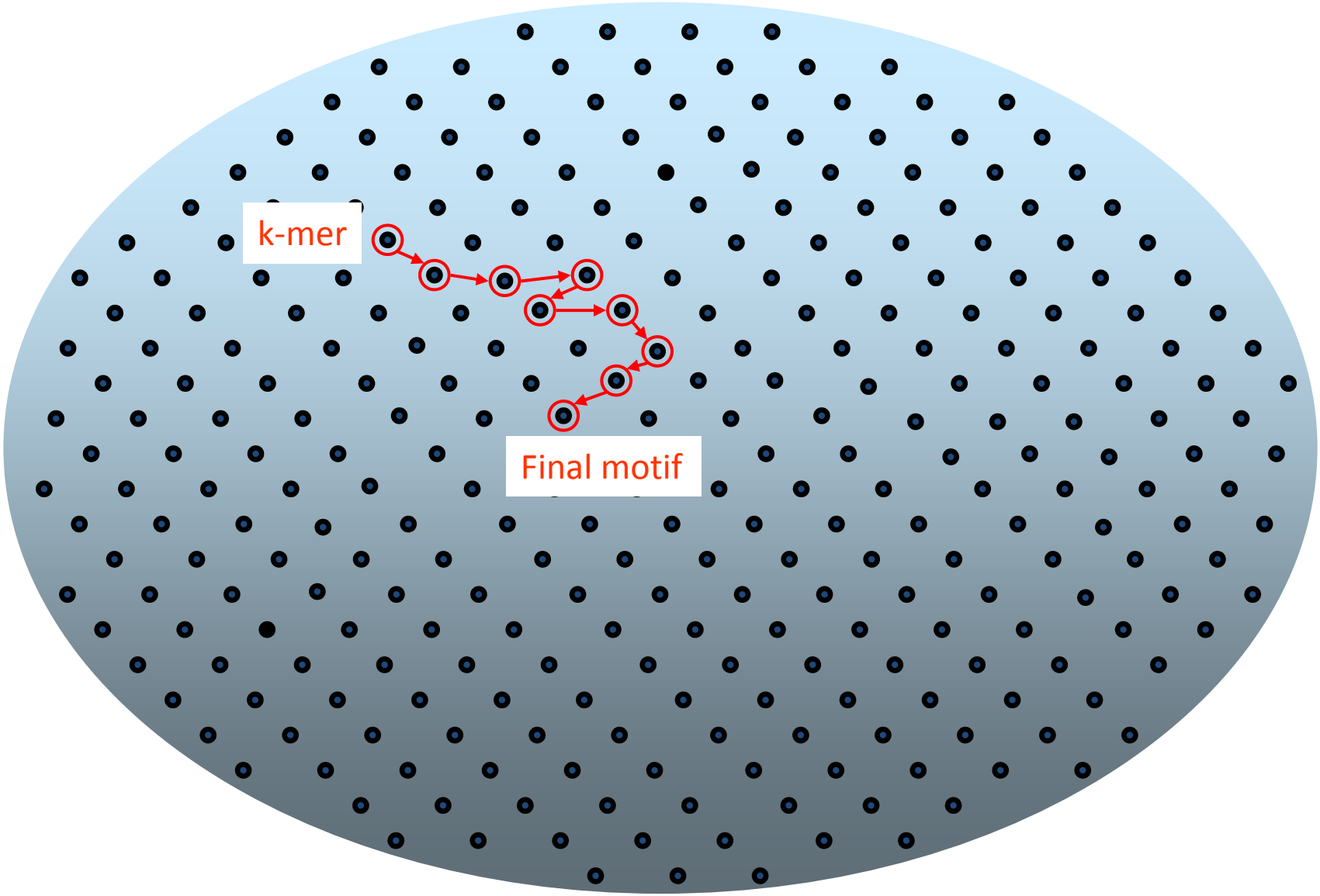
A schematic view of the optimization process



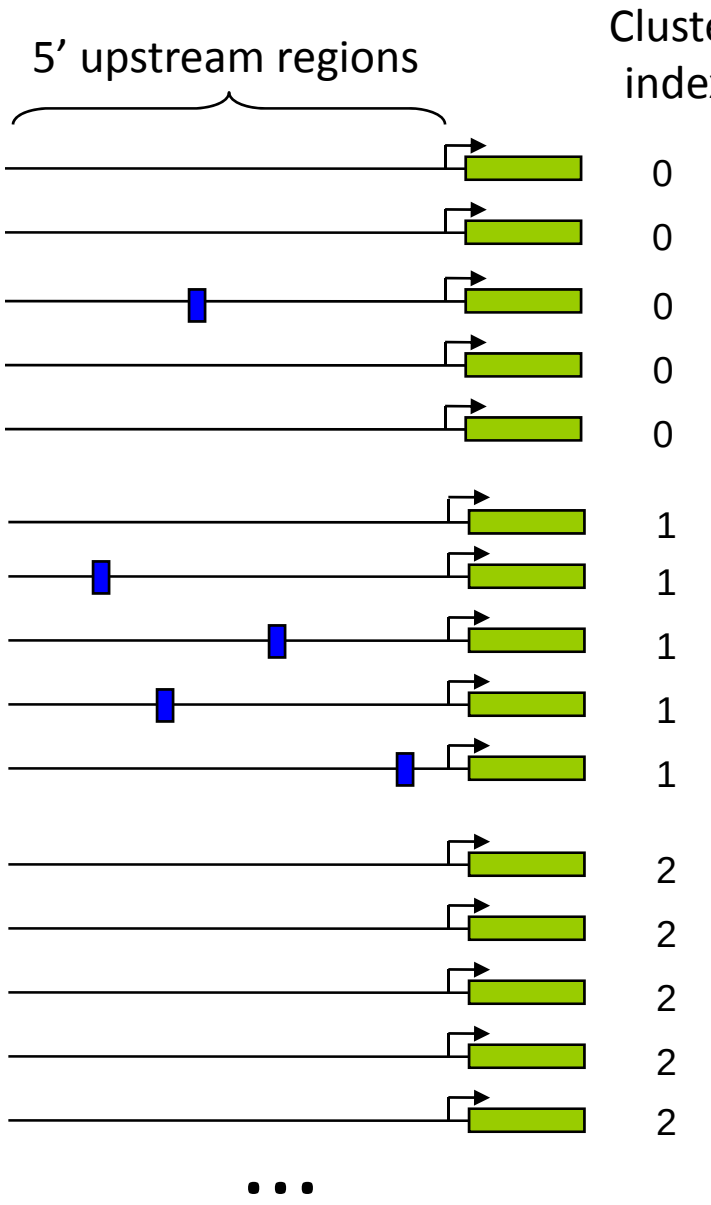
A schematic view of the optimization process



A schematic view of the optimization process



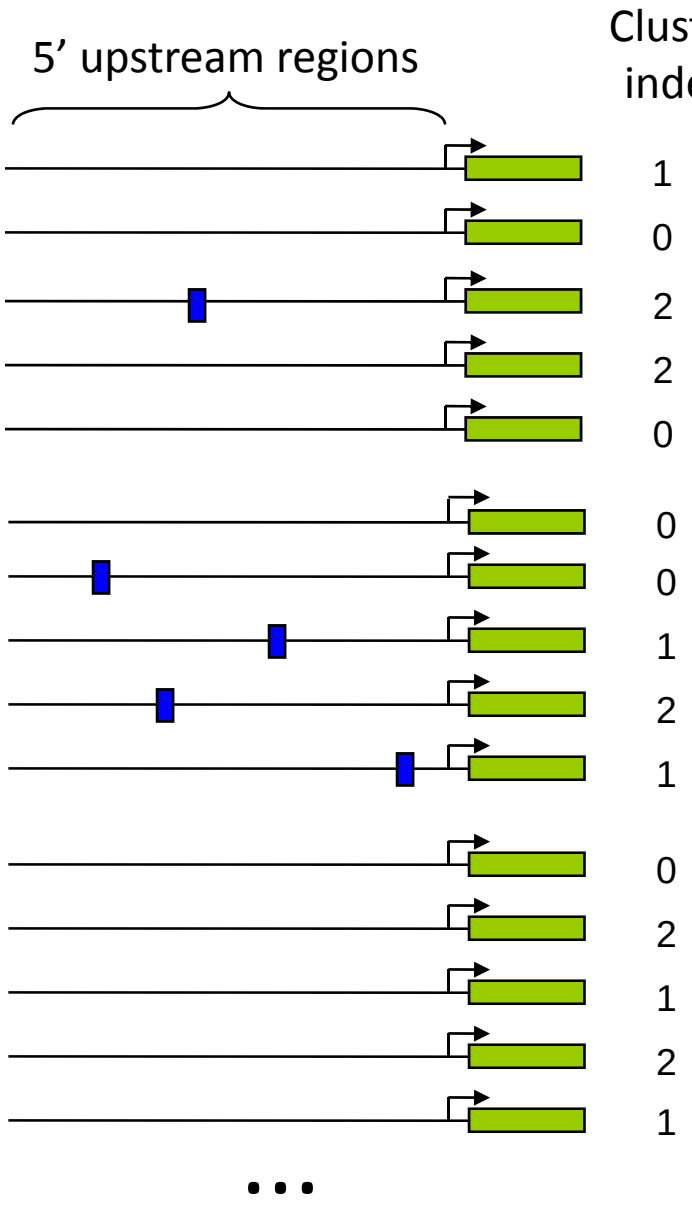
Is a given motif more informative than expected by chance ?



Expression (Cluster Indices)

Motif	0	1	2
Absent	0.27	0.07	0.33
Present	0.07	0.27	0.00

$$I(\text{motif};\text{expression}) = \sum_{i=1}^{2}\sum_{j=1}^{3} P(i,j)\log\frac{P(i,j)}{P(i)P(j)}$$

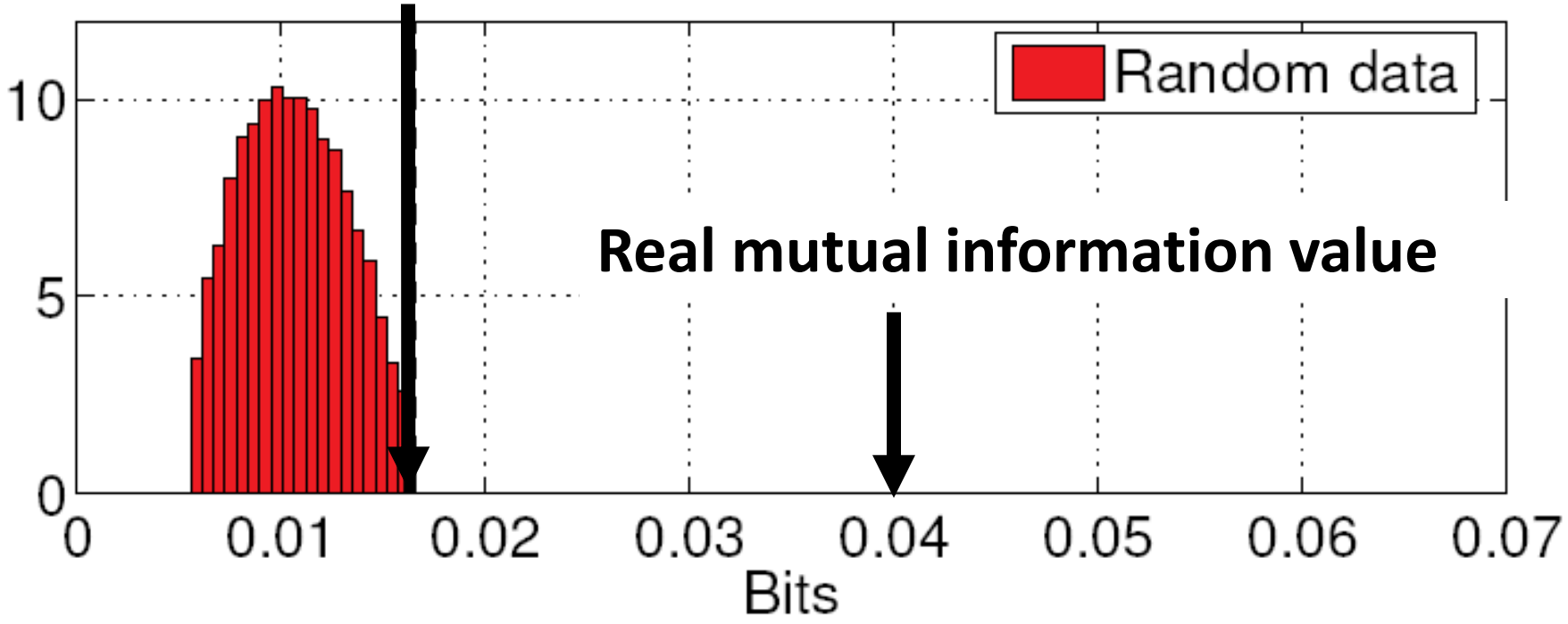


Expression (Cluster Indices)

Motif	0	1	2
Absent	0.16	0.19	0.18
Present	0.19	0.13	0.15

$$I(\text{motif};\text{expression}) = \sum_{i=1}^{2}\sum_{j=1}^{3} P(i,j)\log\frac{P(i,j)}{P(i)P(j)}$$

Maximum of 10,000 expression-shuffled mutual information values

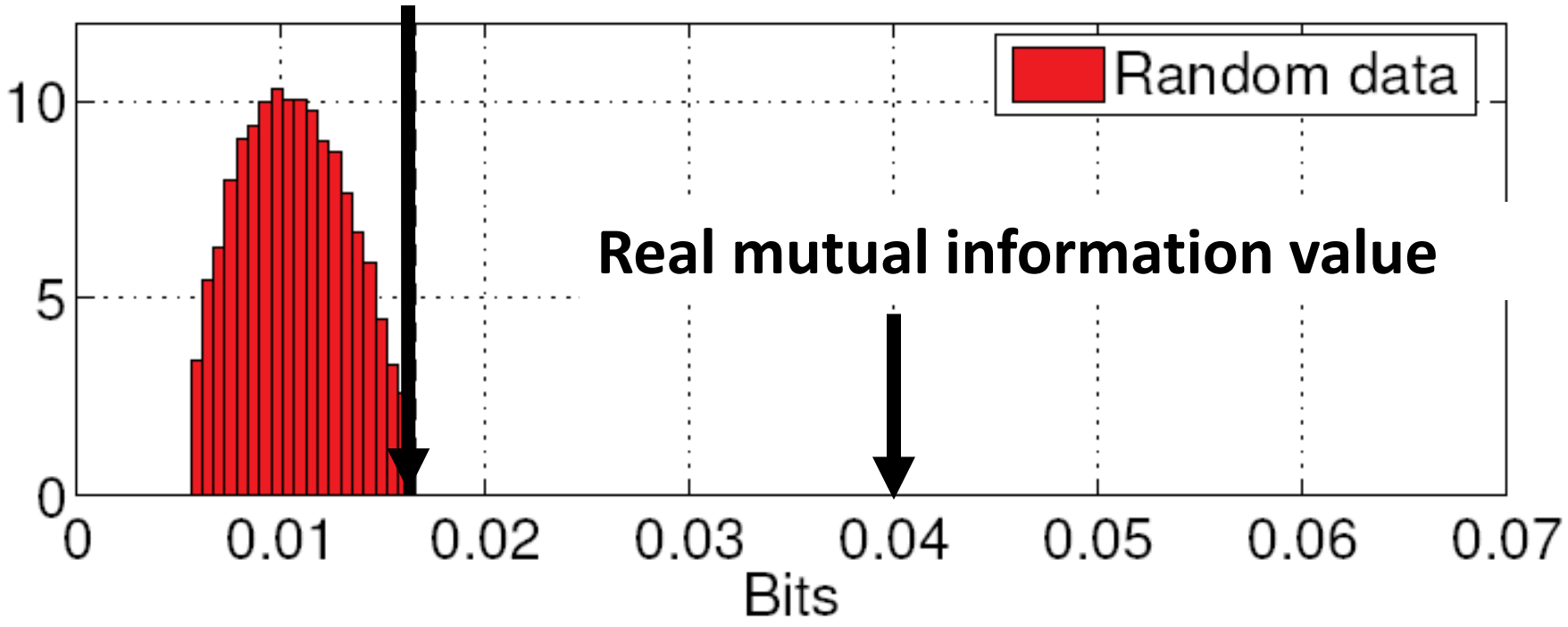


P-value : probability of obtaining by chance a result at least as extreme as observed result

$P(X>=x)$

Maximum of 10,000 expression-shuffled mutual information values

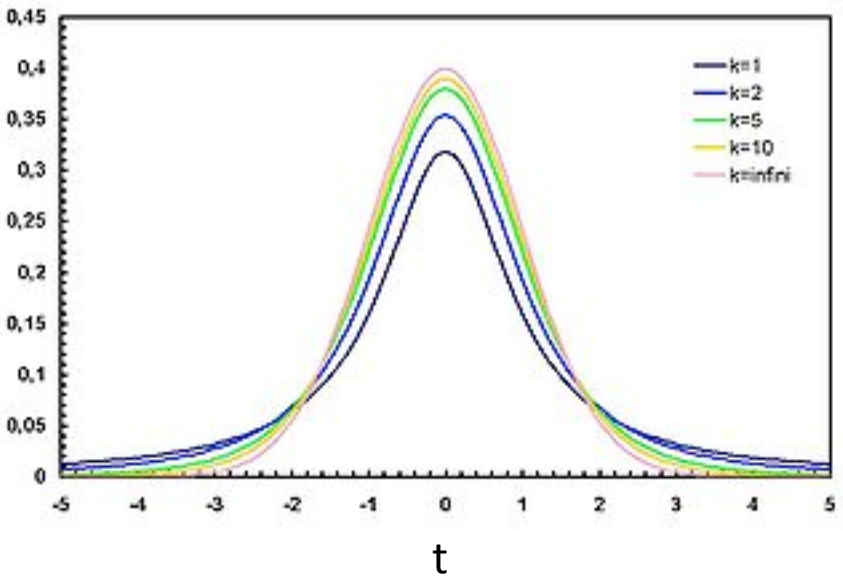
$P<10^{-4}$



Why non-parametric test ?

We don’t know what the null distribution of mutual information is like … depends on sample size, etc.

Null Distribution of T-statistic



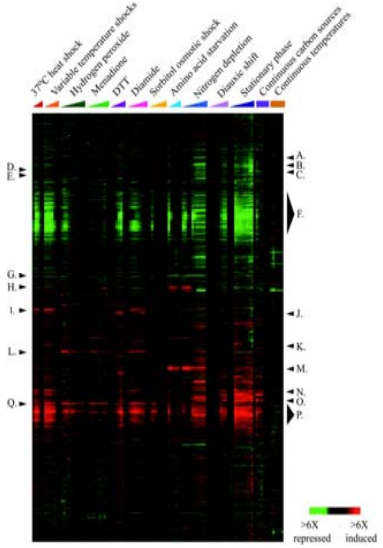
Null Distribution of information values

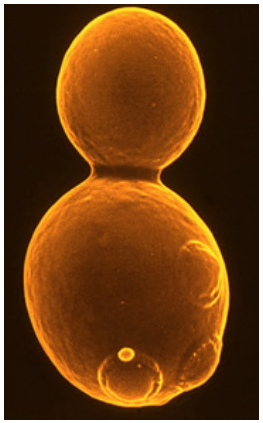
?

So we estimate it using simulation.

Yeast stress gene expression program (Gasch *et al*, 2000)

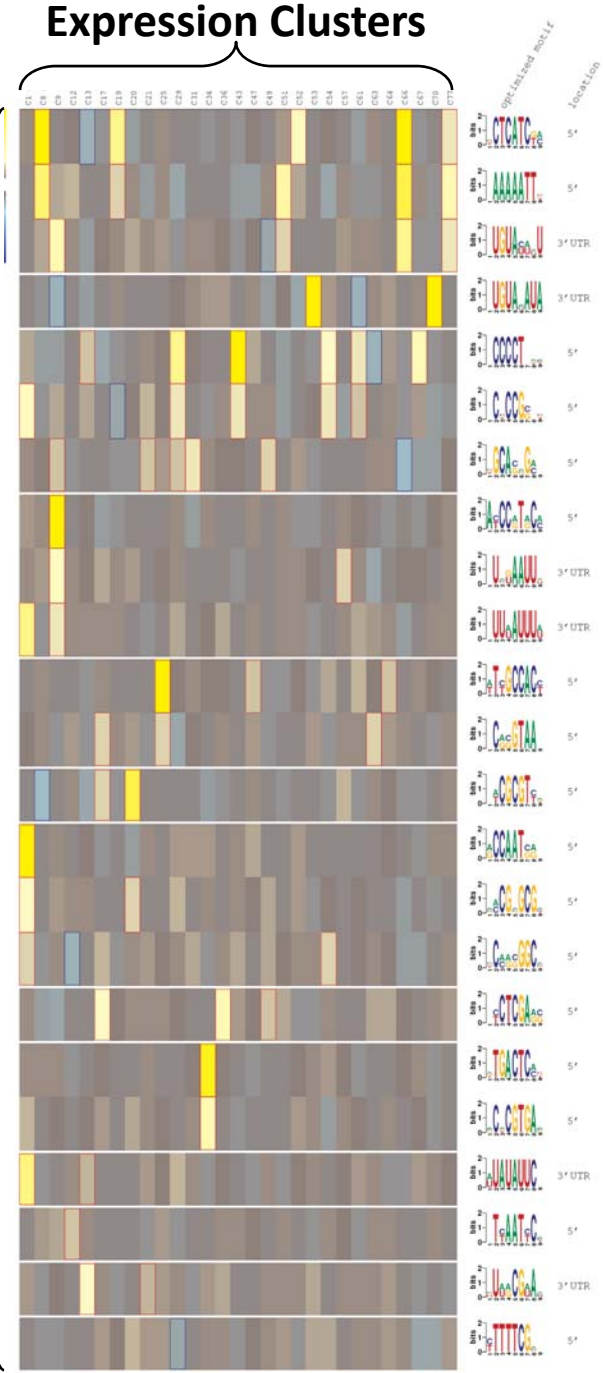
- 173 microarray conditions
- ~ 5,500 genes
- 80 co-expression clusters
- Runtime ~ 1h (standard PC)





Expression Clusters

Predicted Motifs

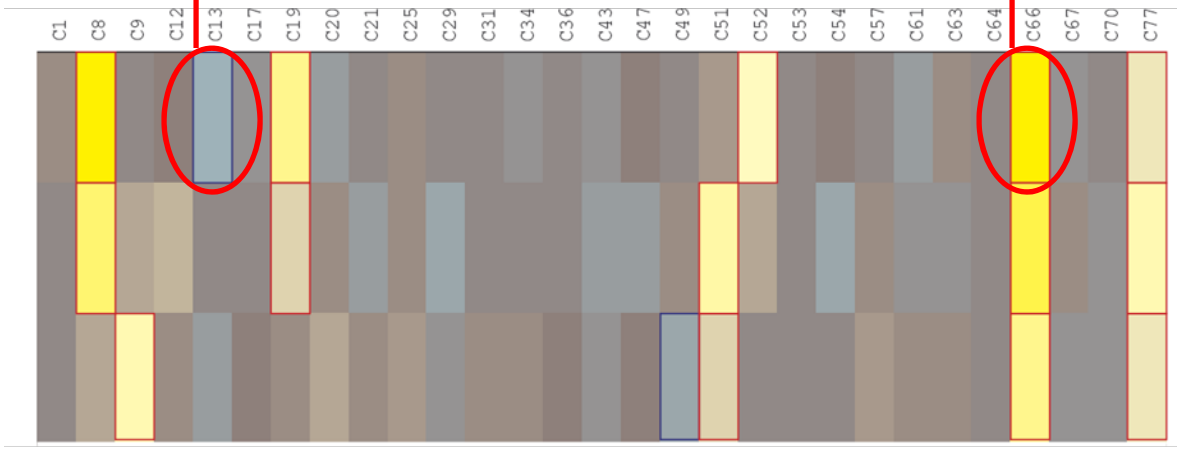


17 motifs in 5’ upstream regions
6 motifs in 3’UTRs

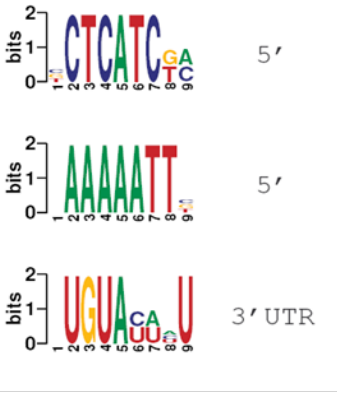
'AC is under-represented in cluster 13 (p<1e-5)

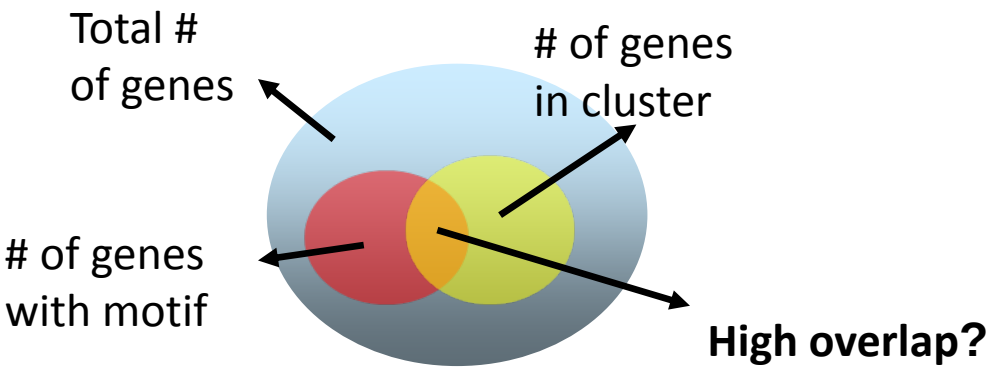
PAC is highly over-represented in cluster 66 (p<1e-20)

Expression Clusters



Motifs

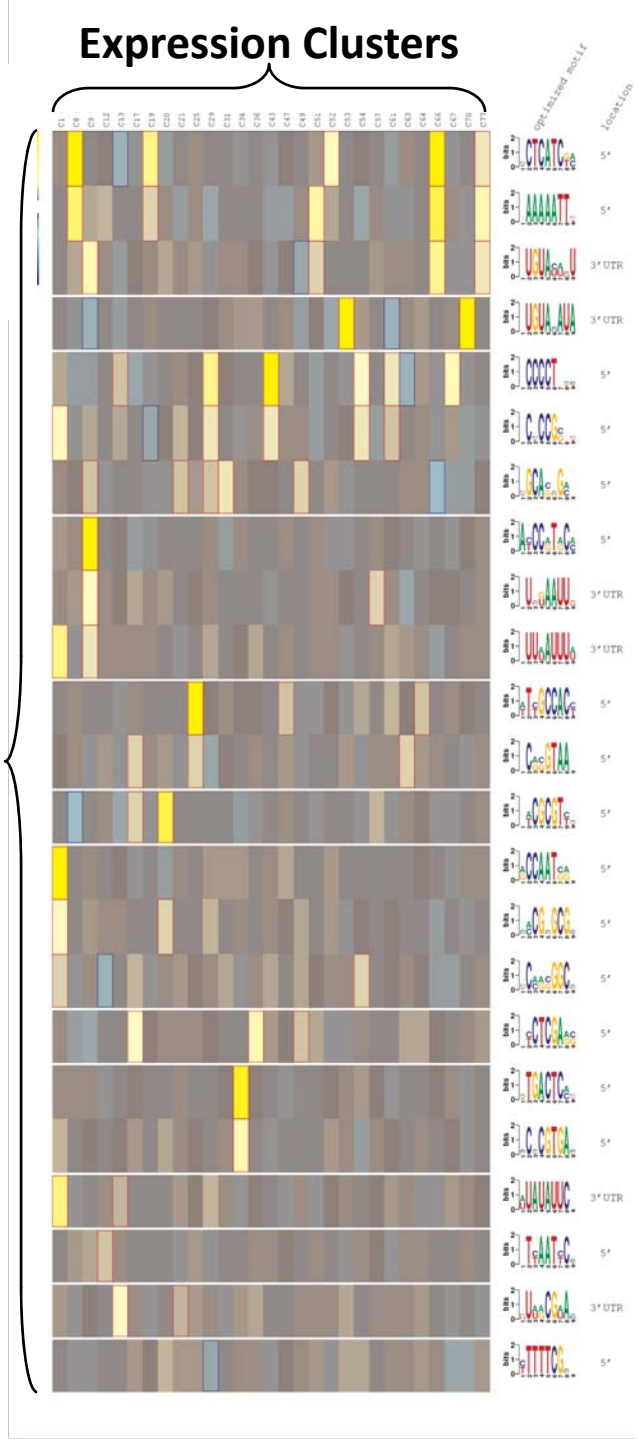




P-value of **over-representation** of a motif in a cluster of genes

$$P(X \geq i) = \sum_{x=i}^{min(s_1,s_2)} \frac{\binom{s_1}{x}\binom{N-s_1}{s_2-x}}{\binom{N}{s_2}}$$

Hypergeometric distribution



17 motifs in 5' upstream regions
6 motifs in 3'UTRs

How many of these motifs are false positives ?

Where do false positives come from ?

- Multiple hypothesis testing (k-mers)
- Overfit by the optimization procedure

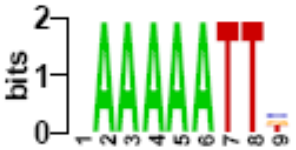
Motif Search Algorithm

Highly informative

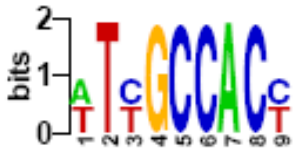
k-mer	MI
CTCATCG	0.0618
TCATCGC	0.0485
AAAATTT	0.0438
GATGAGC	0.0434
AAAAATT	0.0383
ATGAGCT	0.0334
TTGCCAC	0.0322
TGCCACC	0.0298
ATCTCAT	0.0265
...	
...	
ACGCGCG	0.0018
CGACGCG	0.0012
TACGCTA	0.0011
ACCCCCT	0.0010
CCACGGC	0.0009
TTCAAAA	0.0005
AGACGCG	0.0004
CGAGAGC	0.0003
CTTATTA	0.0002

Not informative

MI=0.081



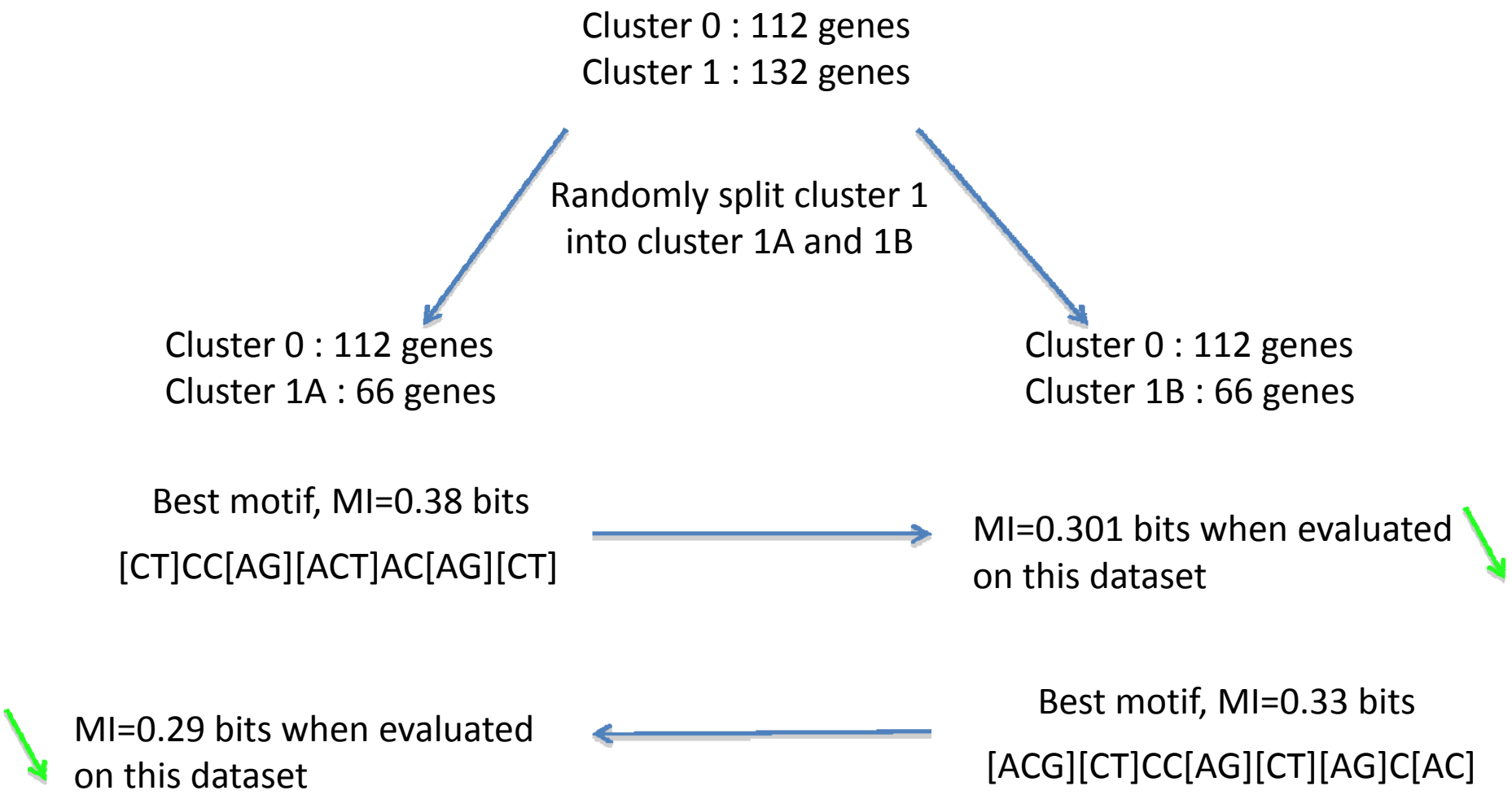
MI=0.045



MI=0.040

...

Does the algorithm overfit motifs to the expression ?

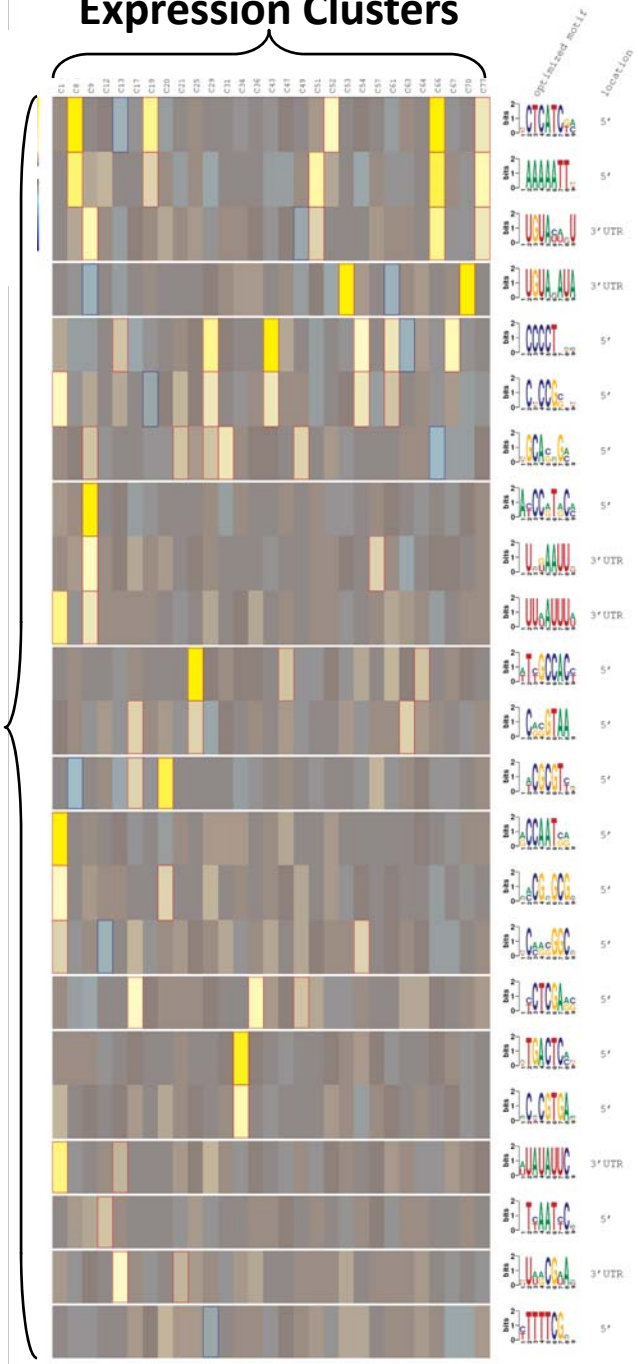


Estimating the false discovery rate

- Run motif discovery algorithm (k-mers+optimization) on random expression profile
- Count how many motifs we get
- Repeat a large number of times, calculate average number of motifs

Expression Clusters

Predicted Motifs



17 motifs in 5’ upstream regions
6 motifs in 3’UTRs

~ 0.05 “motifs” when shuffling the gene labels of the clustering partition

Entropy

$$H(\mathrm{X}) = -\sum_{x} P(x) \log P(x)$$

X	P(X)	
0	0.5	H(X)=1 bit
1	0.5	

X	P(X)	
0	0.8	H(X)=0.72 bits
1	0.2	

X	P(X)	
0	1.0	H(X)=0 bits
1	0.0	

Mutual Information

$$I(\mathrm{X};\mathrm{Y}) = H(Y) - H(Y \mid X)$$

Uncertainty about Y

the amount of uncertainty remaining about Y after X is known