



Bioinformatics for the Identification of Sequences Regulating Gene Transcription

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Overview

Part 1: Prediction of transcription factor binding sites using binding profiles (“Discrimination”)

Part 2: Interrogation of sets of genes to identify mediating transcription factors

Part 3: Detection of novel motifs (TFBS) over-represented in regulatory regions of co-expressed genes (“Discovery”)

Restrictions in Coverage

- Polymerase II driven promoters
 - Generally protein coding genes
- All reference data restricted to activating sequences
 - Information about regulatory elements mediating repression is sparse

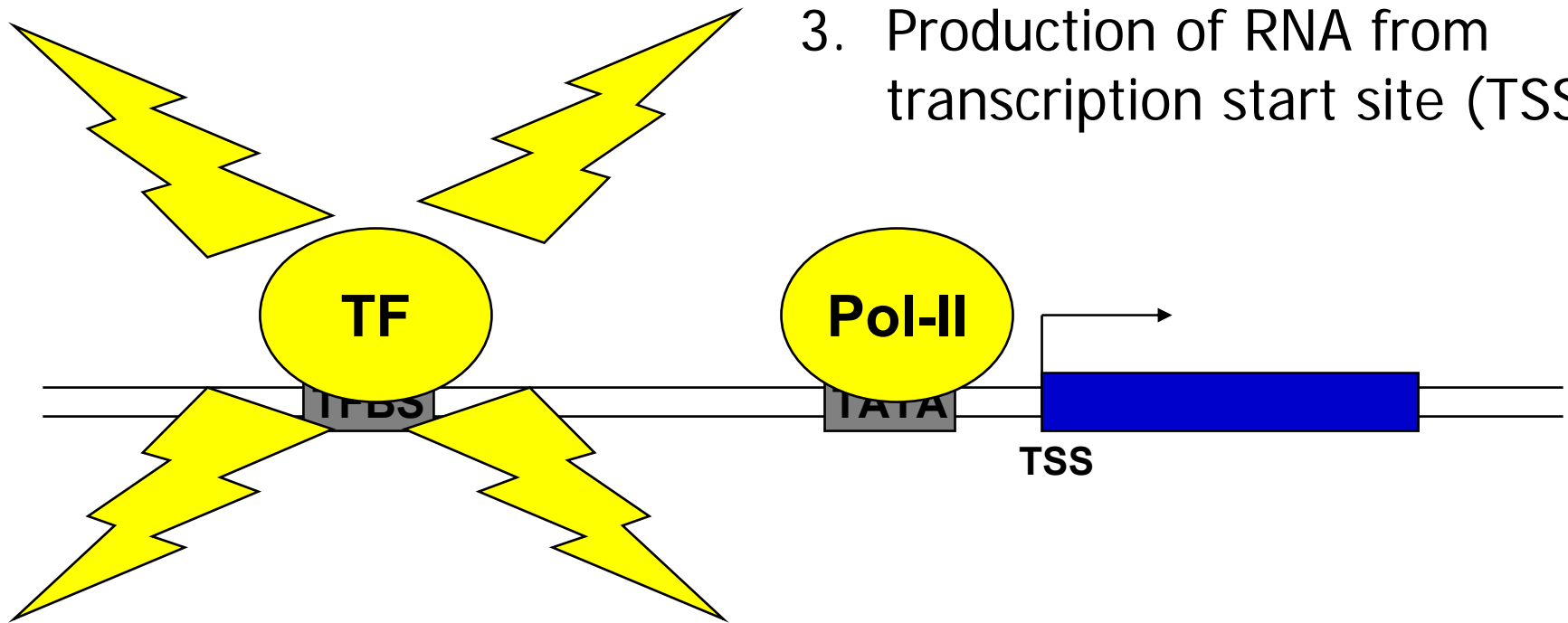
Part 1: Prediction of TF Binding Sites and Regulatory Regions (Discrimination)

Teaching a computer to find TFBS...

Transcription Over-Simplified

Three-step Process:

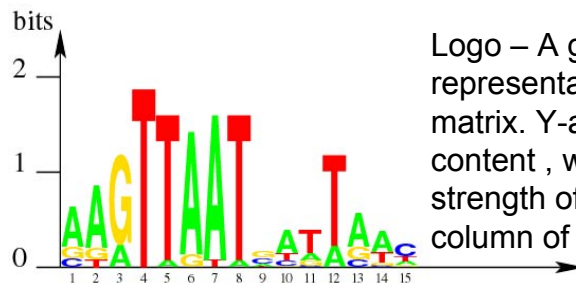
1. TF binds to TFBS (DNA)
2. TF catalyzes recruitment of polymerase II complex
3. Production of RNA from transcription start site (TSS)



Representing Binding Sites for a TF

- A single site
 - AAGTTAATGA
- A set of sites represented as a consensus
 - VDRTWRWWSHD (IUPAC degenerate DNA)
- A matrix describing a set of sites:

A	14	16	4	0	1	19	20	1	4	13	4	4	13	12	3
C	3	0	0	0	0	0	0	0	7	3	1	0	3	1	12
G	4	3	17	0	0	2	0	0	9	1	3	0	5	2	2
T	0	2	0	21	20	0	1	20	1	4	13	17	0	6	4



Logo – A graphical representation of frequency matrix. Y-axis is information content, which reflects the strength of the pattern in each column of the matrix

Set of binding sites

AAGTTAATGA
 CAGTTAATAA
 GAGTTAAACA
 CAGTTAATTA
 GAGTTAATAA
 CAGTTATTCA
 GAGTTAATAA
 CAGTTAATCA
 AGATTAAAGA
 AAGTTAACGA
 AGGTTAACGA
 ATGTTGATGA
 AAGTTAATGA
 AAGTTAACGA
 AAATTAATGA
 GAGTTAATGA
 AAGTTAATCA
 AAGTTGATGA
 AAATTAATGA
 ATGTTAATGA
 AAGTAAATGA
 AAGTTAATGA
 AAGTTAATGA
 AAATTAATGA
 AAGTTAATGA
 AAGTTAATGA
 AAGTTAATGA
 AAGTTAATGA
 AAGTTAATGA
 AAGTTAATGA

Conversion of PFM to Position Specific Scoring Matrix (PSSM)

Add the following features to the matrix profile:

1. Correct for nucleotide frequencies in genome
2. Weight for the confidence (depth) in the pattern
3. Convert to log-scale probability for easy arithmetic

<i>pfm</i>						<i>pssm</i>						
A	5	0	1	0	0	$\text{Log} \left(\frac{f(b,i) + s(n)}{p(b)} \right)$	A	1.6	-1.7	-0.2	-1.7	-1.7
C	0	2	2	4	0		C	-1.7	0.5	0.5	1.3	-1.7
G	0	3	1	0	4		G	-1.7	1.0	-0.2	-1.7	1.3
T	0	0	1	1	1		T	-1.7	-1.7	-0.2	-0.2	-0.2

TGCTG = 0.9



JASPAR: AN OPEN-ACCESS DATABASE OF TF BINDING PROFILES

(Transfac database is a commercial alternative)

The Good...

- Tronche (1997) tested 50 predicted HNF1 TFBS using an in vitro binding test and found that 96% of the predicted sites were bound!
- Stormo and Fields (1998) found in detailed biochemical studies that the best PSSMs produce binding site prediction scores highly correlated with in vitro binding energy

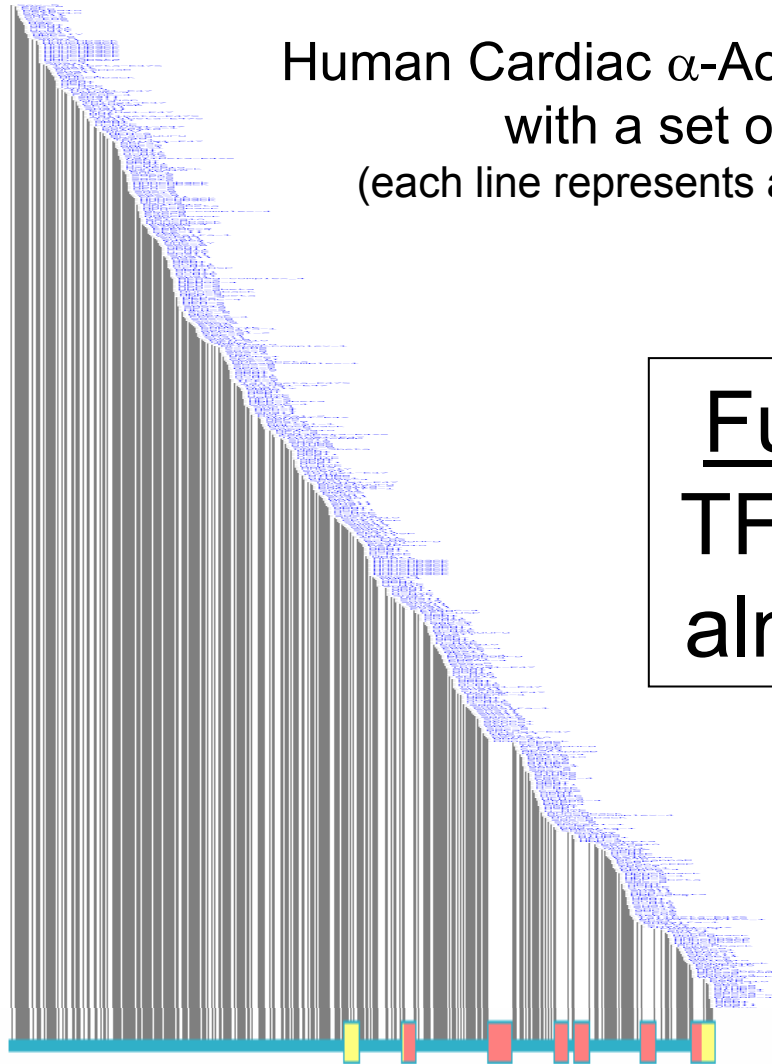
...the Bad...

- Fickett (1995) found that a profile for the myoD TF made predictions at a rate of 1 per ~500bp of human DNA sequence
 - This corresponds to an average of 20 sites / gene (assuming 10,000 bp as average gene size)

...and the Ugly!

Human Cardiac α -Actin gene analyzed
with a set of profiles
(each line represents a TFBS prediction)

Futility Conjecture:
TFBS predictions are
almost always wrong

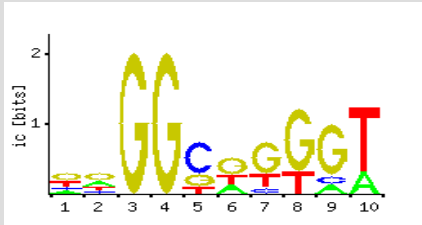


Red boxes are protein coding exons -
TFBS predictions excluded in this analysis

Detecting binding sites in a single sequence

Scanning a sequence against a PWM

Sp1



ACCCTCCCCAGGGGCGGGGGGCGGTGGCCAGGACGGTAGCTCC

A	[-0.2284	0.4368	-1.5	-1.5	-1.5	0.4368	-1.5	-1.5	-0.2284	0.4368]
C	[-0.2284	-0.2284	-1.5	-1.5	1.5128	-1.5	-0.2284	-1.5	-0.2284	-1.5]
G	[1.2348	1.2348	2.1222	2.1222	0.4368	1.2348	1.5128	1.7457	1.7457	-1.5]
T	[0.4368	-0.2284	-1.5	-1.5	-0.2284	0.4368	0.4368	0.4368	-1.5	1.7457]

Abs_score = 13.4 (sum of column scores)

Calculating the relative score

A	[-0.2284	0.4368	-1.5	-1.5	-1.5	0.4368	-1.5	-1.5	-0.2284	0.4368]
C	[-0.2284	-0.2284	-1.5	-1.5	1.5128	-1.5	-0.2284	-1.5	-0.2284	-1.5]
G	[1.2348	1.2348	2.1222	2.1222	0.4368	1.2348	1.5128	1.7457	1.7457	-1.5]
T	[0.4368	-0.2284	-1.5	-1.5	-0.2284	0.4368	0.4368	0.4368	-1.5	1.7457]

Max_score = 15.2 (sum of highest column scores)

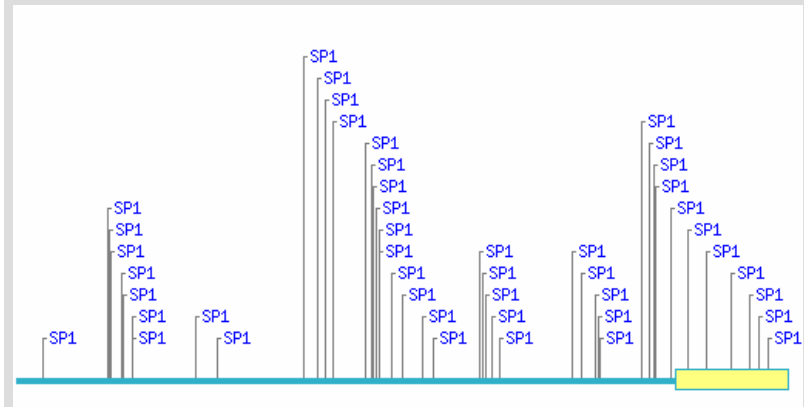
A	[-0.2284	0.4368	-1.5	-1.5	-1.5	0.4368	-1.5	-1.5	-0.2284	0.4368]
C	[-0.2284	-0.2284	-1.5	-1.5	1.5128	-1.5	-0.2284	-1.5	-0.2284	-1.5]
G	[1.2348	1.2348	2.1222	2.1222	0.4368	1.2348	1.5128	1.7457	1.7457	-1.5]
T	[0.4368	-0.2284	-1.5	-1.5	-0.2284	0.4368	0.4368	0.4368	-1.5	1.7457]

Min_score = -10.3 (sum of lowest column scores)

$$\text{Rel_score} = \frac{\text{Abs_score} - \text{Min_score}}{\text{Max_score} - \text{Min_score}} \cdot 100\%$$

$$= \frac{13.4 - (-10.3)}{15.2 - (-10.3)} \cdot 100\% = 93\%$$

Scanning 1300 bp of human insulin receptor gene with Sp1 at rel_score threshold of 75%



Ouch.

Observations

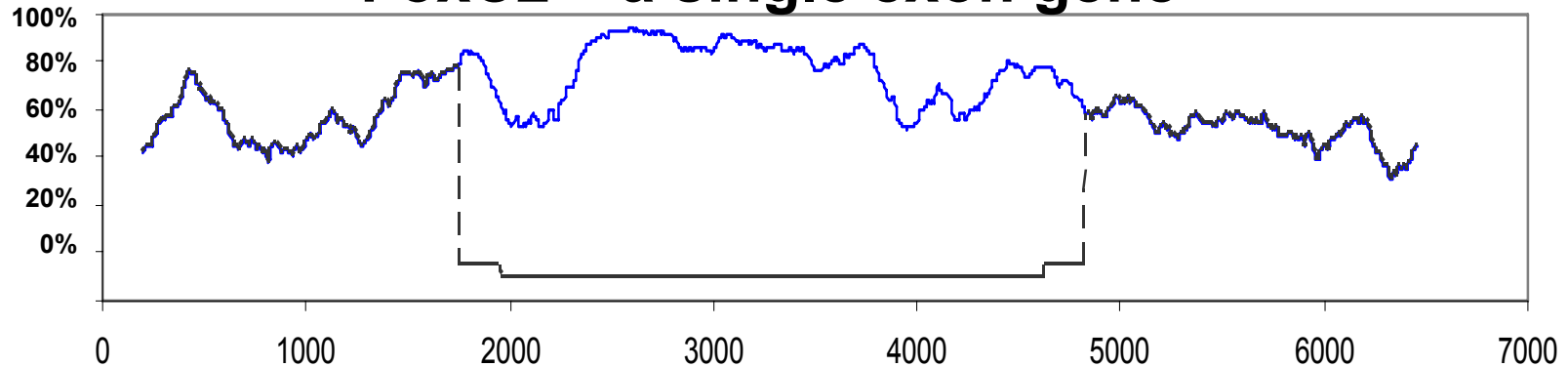
- PSSMs accurately reflect in vitro binding properties of DNA binding proteins
- High-scoring “binding sites” occur at a rate far too frequent to reflect in vivo function
- Bioinformatics methods that use PSSMs for binding site studies must incorporate additional information to enhance specificity

Using Phylogenetic Footprinting to Improve TFBS Discrimination

70,000,000 years of evolution
can reveal regulatory regions

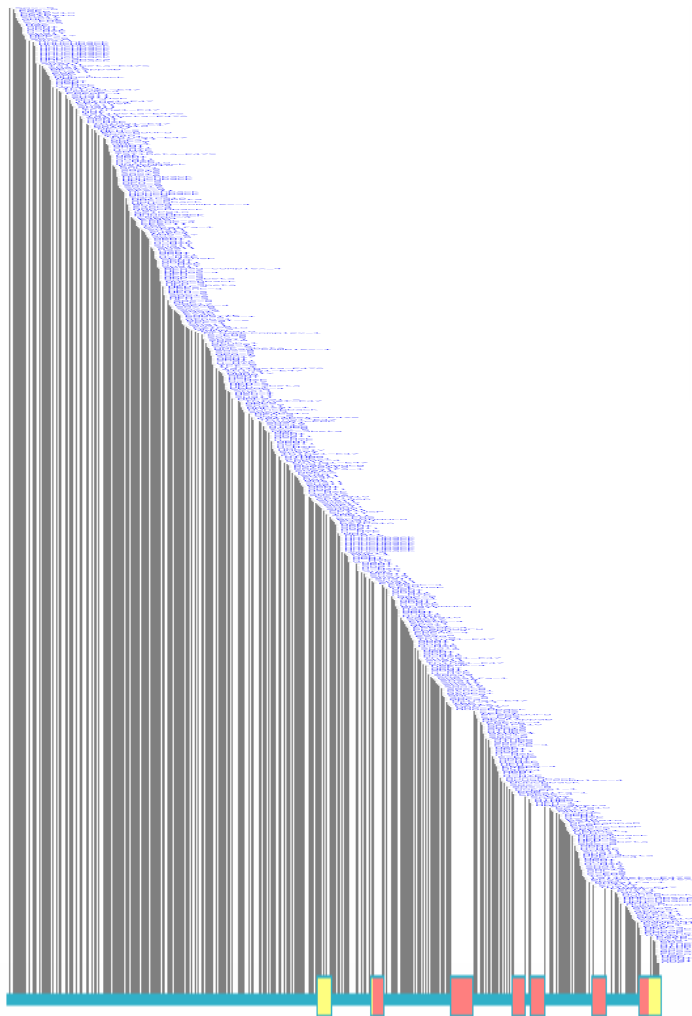
Phylogenetic Footprinting

FoxC2 – a single exon gene

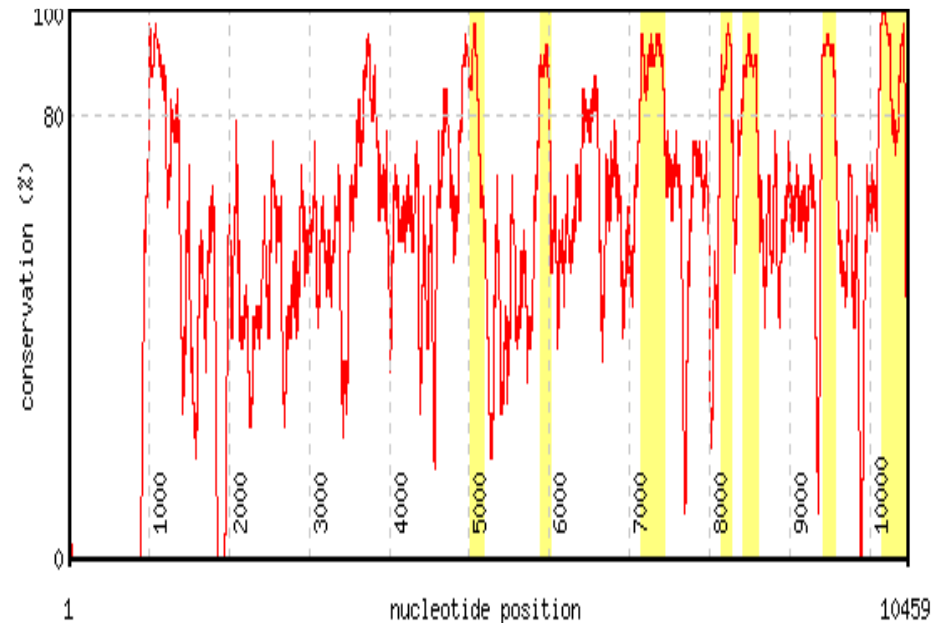


- Align orthologous gene sequences (e.g. LAGAN)
- For first window of 100 bp, of sequence#1, determine the % with identical match in sequence#2
 - Step across the first sequence, recording the percentage of identical nucleotides in each window
- Observe that single exon contains a region of high identity that corresponds to the ORF, with lower identity in the 5' and 3' UTRs
- Additional conserved region could be regulatory regions

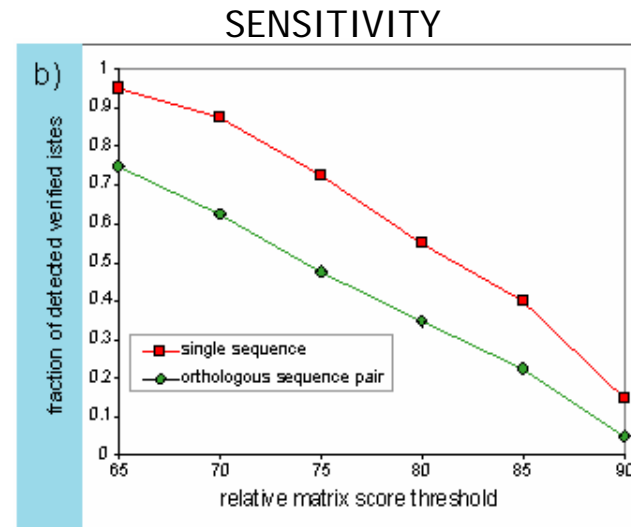
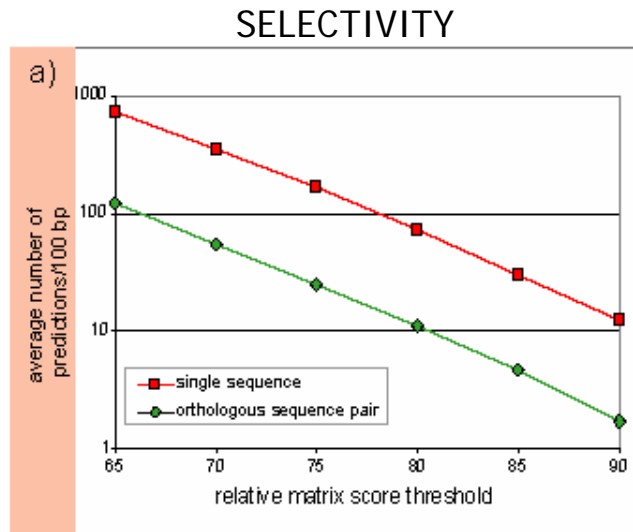
Phylogenetic Footprinting Dramatically Reduces False Predictions



Actin, alpha cardiac



TFBS Prediction with Human & Mouse Pairwise Phylogenetic Footprinting

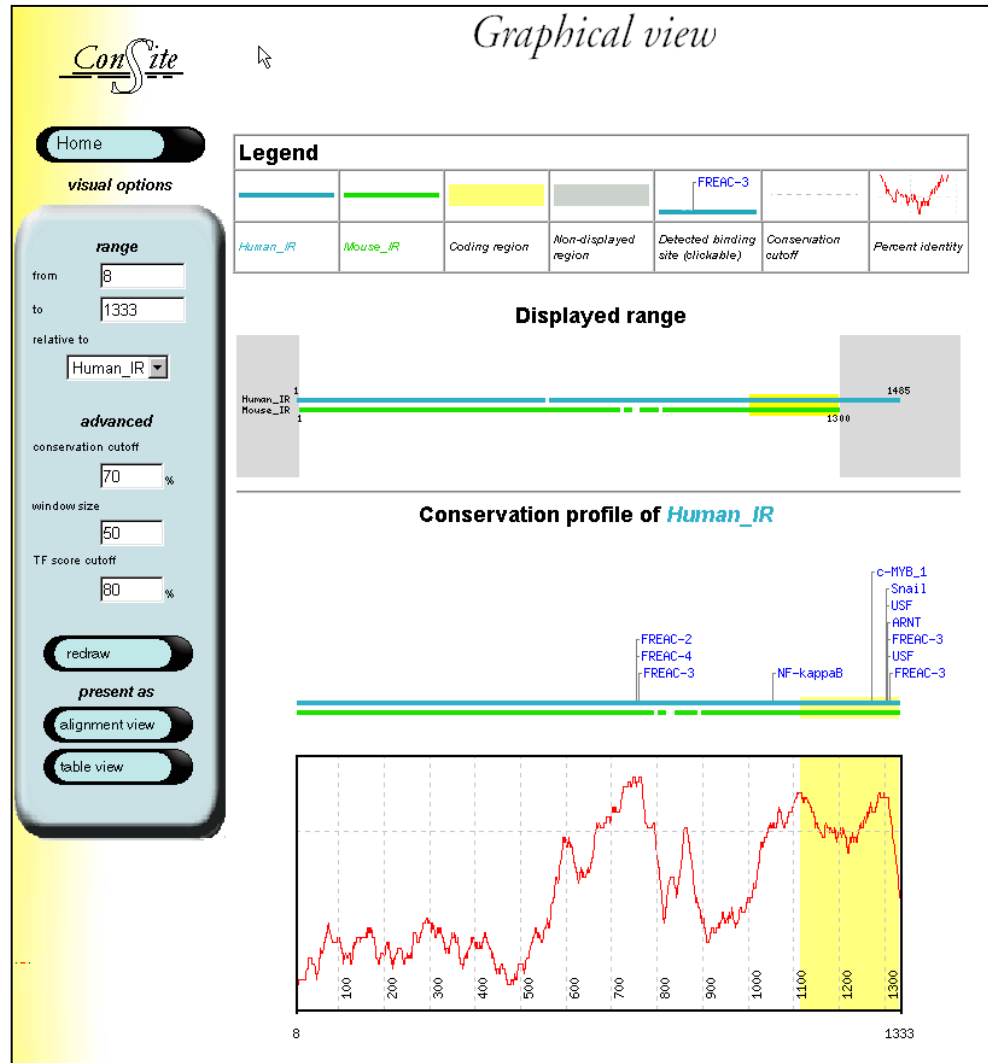


- Testing set: 40 experimentally defined sites in 15 well studied genes (Replicated with 100+ site set)
- 75-80% of defined sites detected with conservation filter, while only 11-16% of total predictions retained

1kbp beta-globin promoter screened with footprinting



ConSite



OnLine Resources for Phylogenetic Footprinting

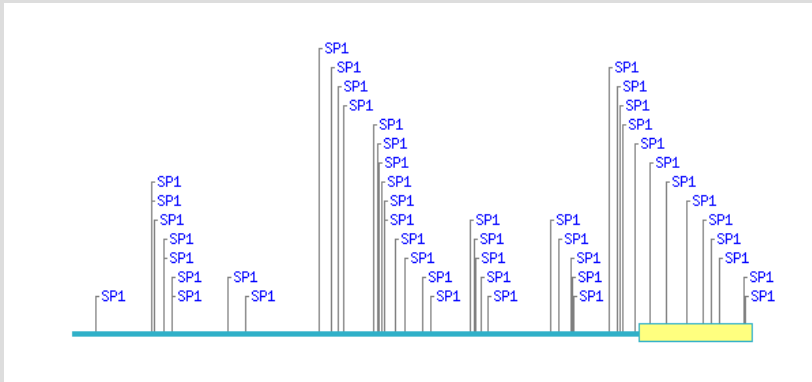
- Linked to TFBS
 - ConSite
 - rVISTA
 - Footprinter
- Visualization
 - Sockeye
 - Vista Browser
 - PipMaker
- Alignments
 - Blastz
 - Lagan/mLAGAN
 - Avid
 - ORCA

Multi-species Phylogenetic Footprinting

- In bioinformatics we hate to ignore useful information...
 - Pairwise comparisons do not take full advantage of the growing set of sequenced genomes
- New algorithms (e.g. Monkey) weight TFBS predictions based on retention over a branch of a species tree
 - Method is compute intensive, as each predicted TFBS is assessed against all other predictions
- Not clear what the relative benefits of multi-species methods will be...
 - Some suggestions that the best pairwise comparison gives similar results to a multi-species comparison

Analysis of TFBS with Phylogenetic Footprinting

Scanning a single sequence

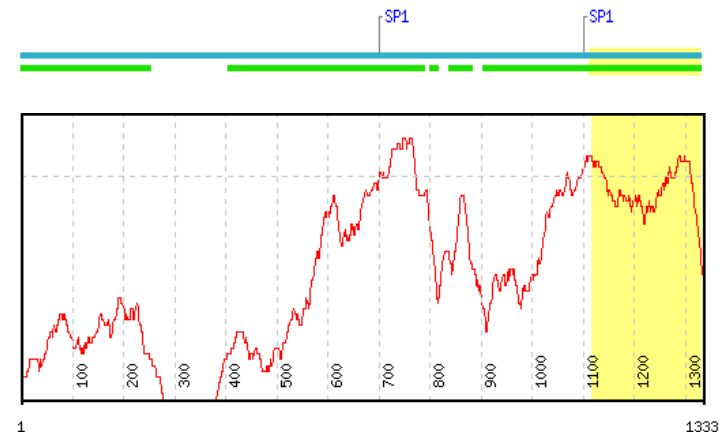


Low specificity of profiles:

- too many hits
- great majority not biologically significant

Scanning a pair of orthologous sequences for conserved patterns in conserved sequence regions

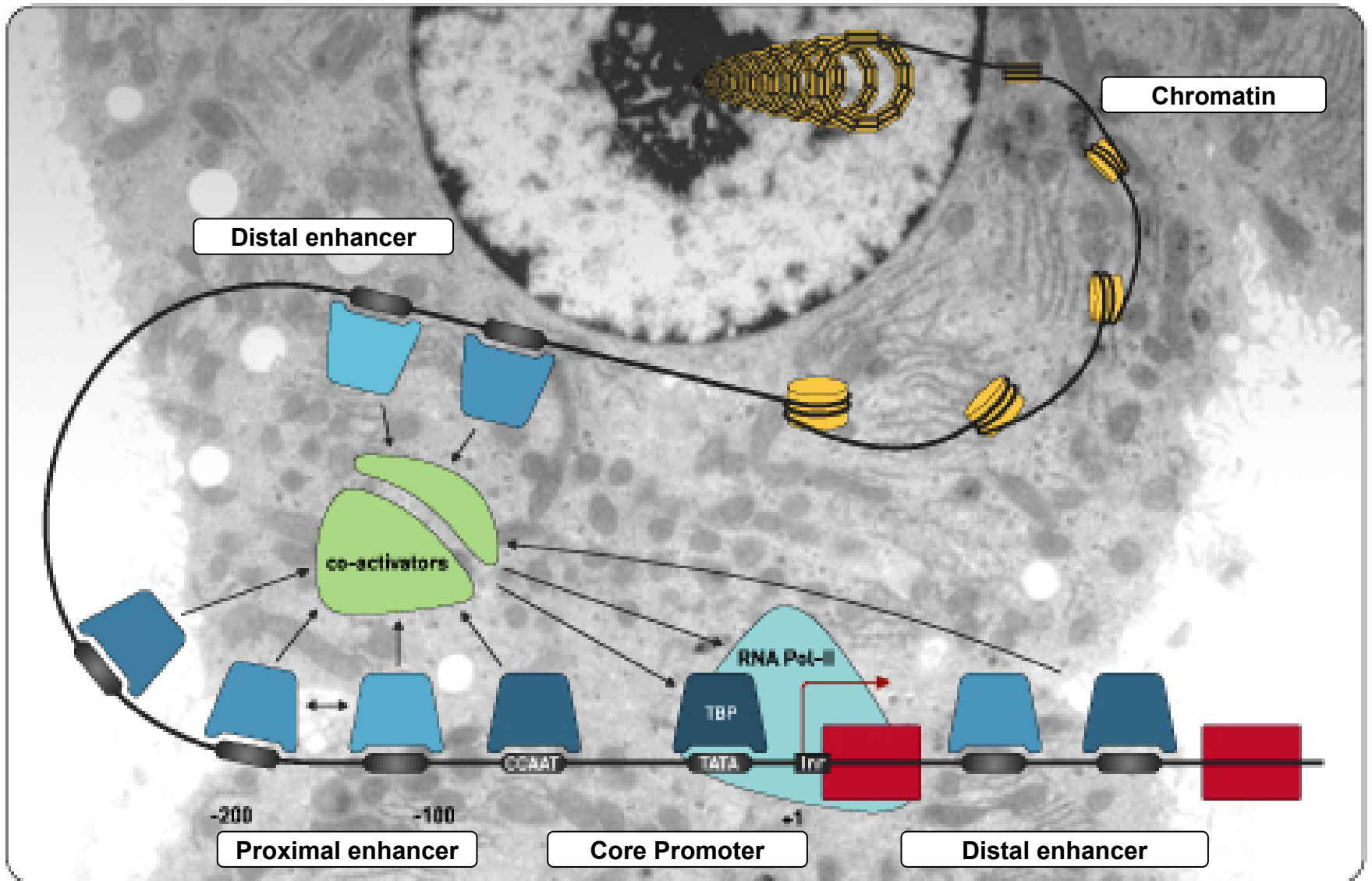
A dramatic improvement in the percentage of biologically significant detections



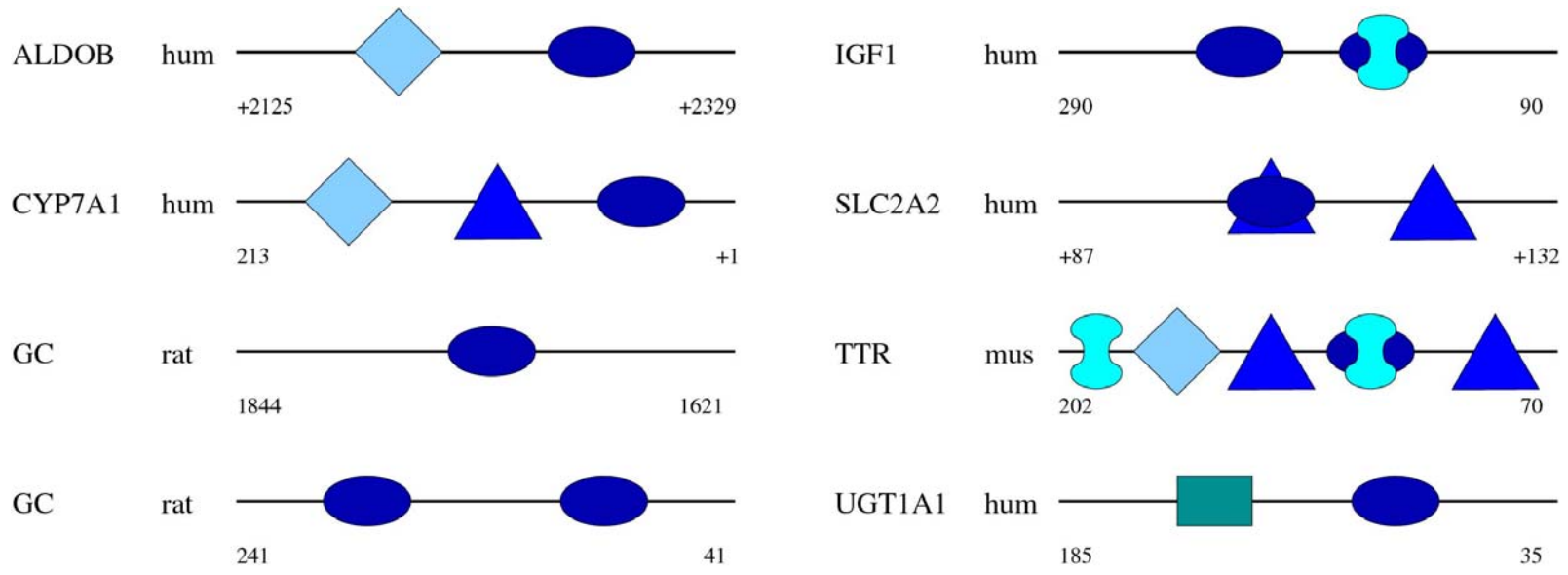
Discrimination of Regulatory Modules

TFs do NOT act in isolation
(THIS SECTION IS BRIEF DUE TO TIME CONSTRAINTS)

Complexity in Transcription



Known *cis*-regulatory modules (CRMs) for specific expression in hepatocytes



HNF1



HNF3



HNF4



C/EBP



Sp1

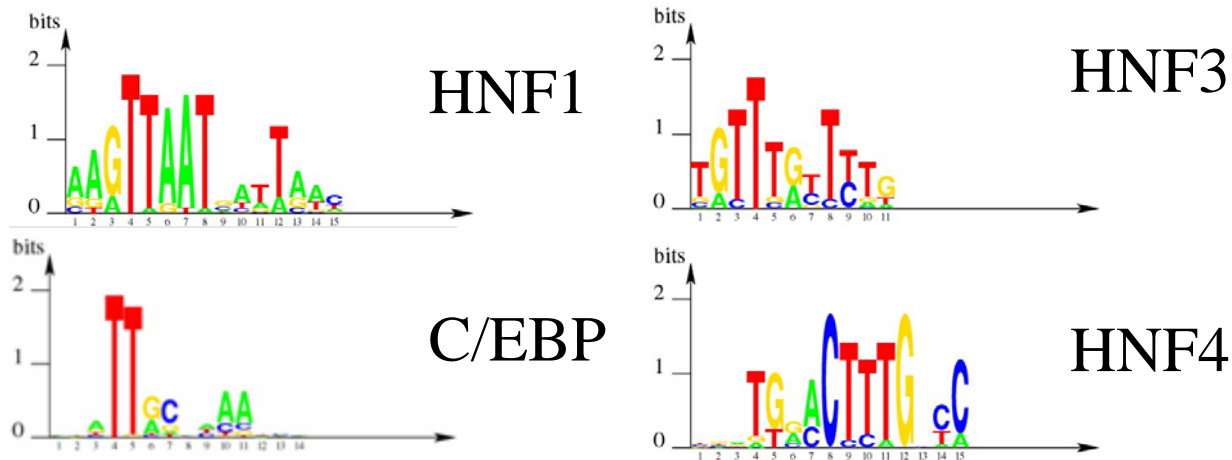


Detecting Clusters of TFBS

- GOAL: Given a set of profiles for TFs known (or hypothesized) to act together, teach computer to find clusters of TFBS
- Trained Methods
 - Sufficient examples of real clusters to establish weights on the relative importance of each TF
- Statistical Over-Representation of Combinations
 - Binding profiles available for a set of biologically motivated TFs
 - Usually confounded by the non-random properties of genomic sequences
 - Requires substantial effort to model local sequence properties in order to determine significance

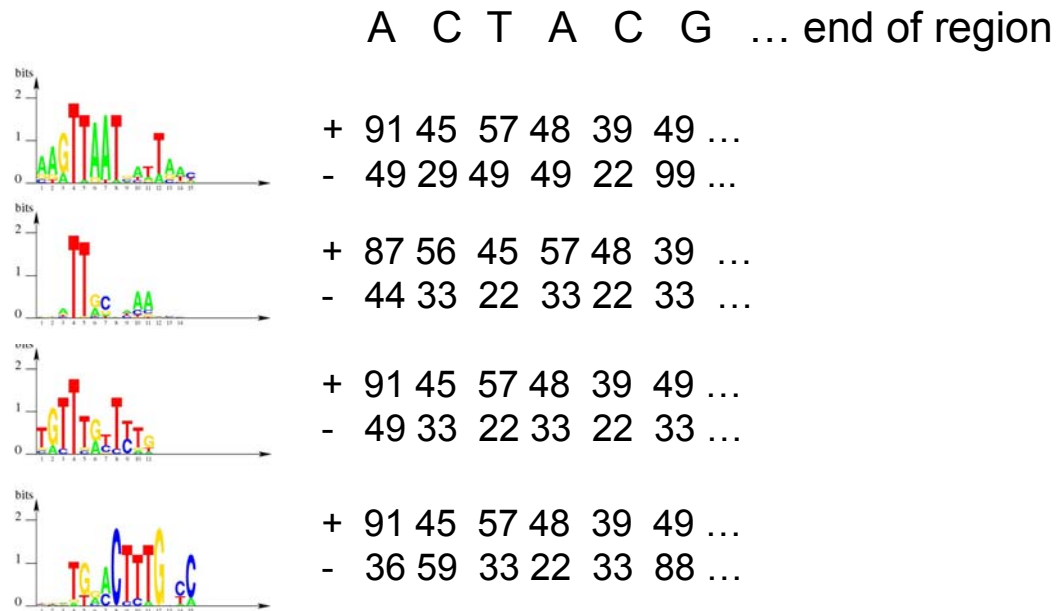
Building a trained model (1)

Step 1: Obtain a set of PSSMs for the mediating TFs



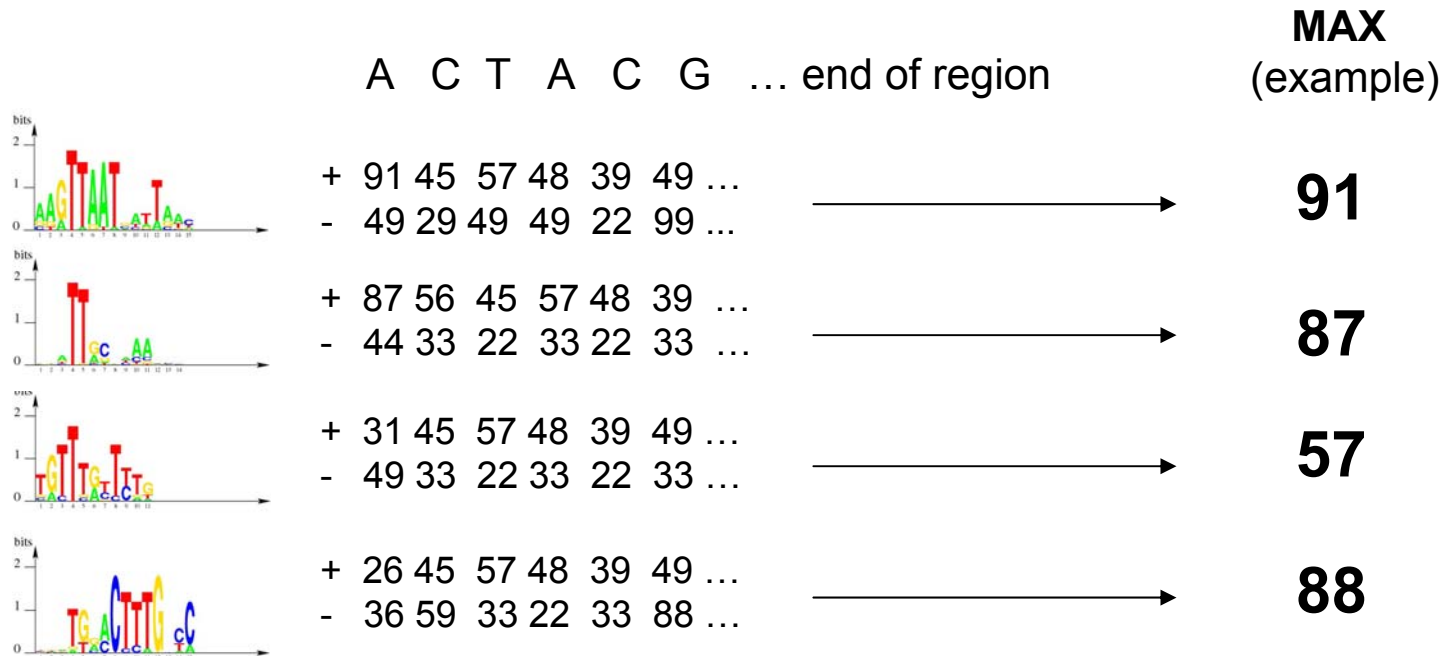
Building a trained model (2)

Step 2: Score all possible sites in each reference sequence with each profile (don't forget second strand)



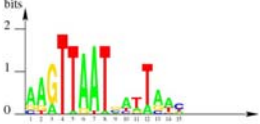
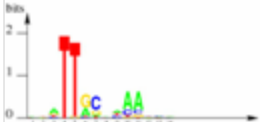
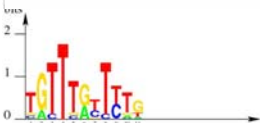
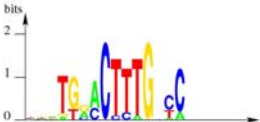
Building a trained model (3)

Step 3: Filter the scores (many possible approaches at this stage)



Building a trained model (4)

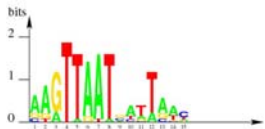
Step 4: Obtain scores for each sequence...

	HEPATOCYTE MODULES				NEGATIVE CONTROLS			
	MAX_{H1}	MAX_{H2}	...	MAX_{Hn}	MAX_{C1}	MAX_{C2}	MAX_{Cn}
	91	75	...	82	45	56	...	87
	87	34	...	56	33	44	...	28
	57	44	...	33	48	37	...	55
	88	44	...	27	22	33	...	44

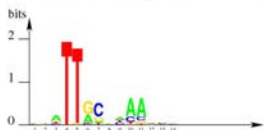
Building a trained model (5)

Step 5: Statistically determine a weight to place upon the scores of each profile...

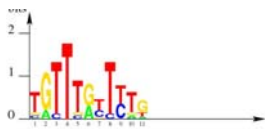
	HEPATOCYTE MODULES				NEGATIVE CONTROLS				WEIGHTS
	MAX_{H1}	MAX_{H2}	...	MAX_{Hn}	MAX_{C1}	MAX_{C2}	MAX_{Cn}	



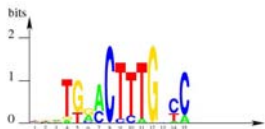
91	75	...	82	45	56	...	87	.1
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87	34	...	56	33	44	...	28	.2
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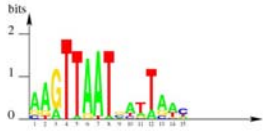
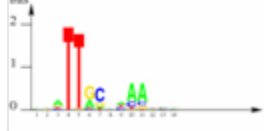
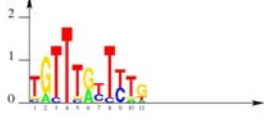
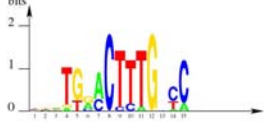
57	44	...	33	48	37	...	55	0
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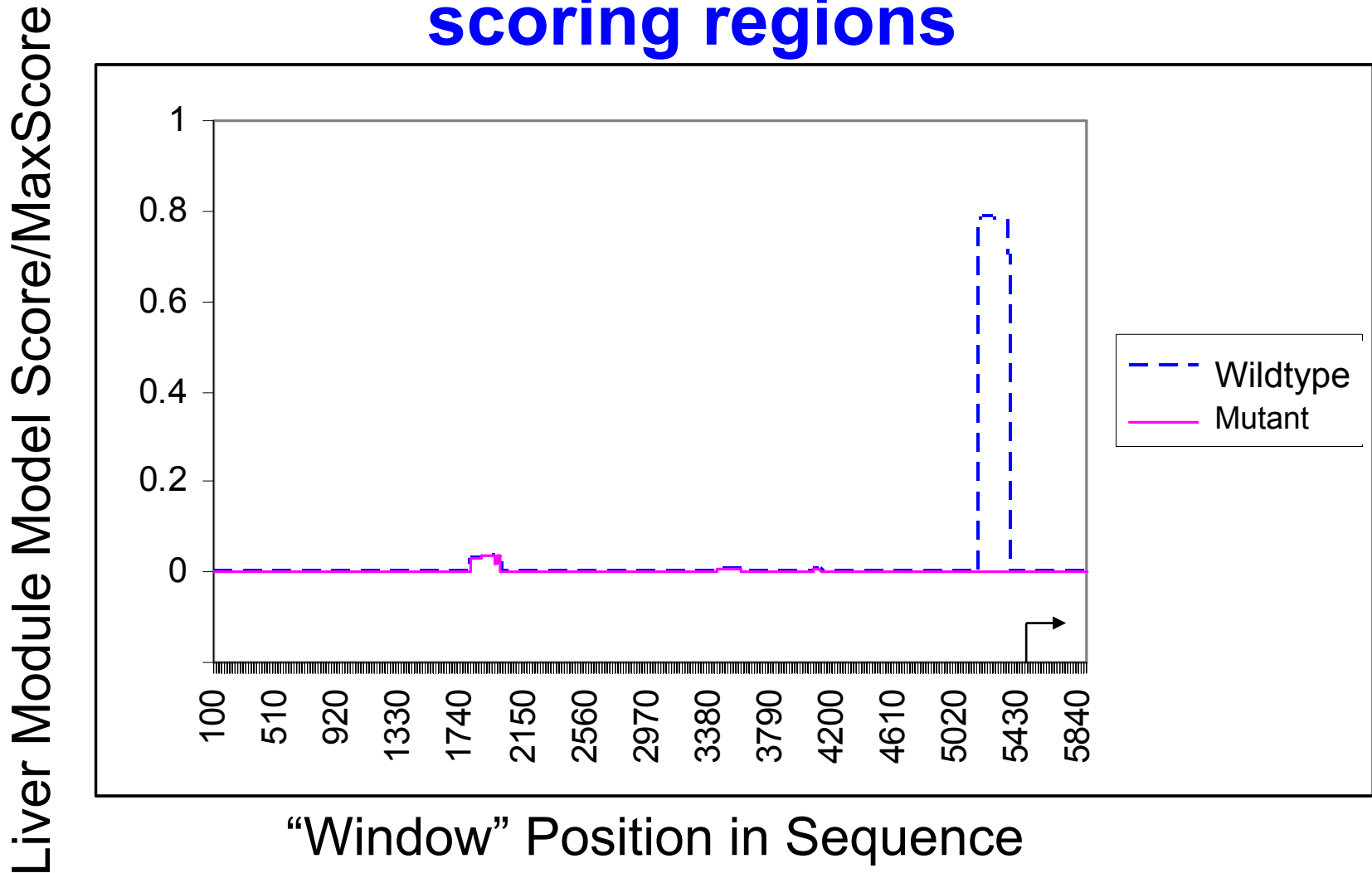
88	44	...	27	22	33	...	44	.2
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Building a trained model (6)

Step 6: Calculate scores for test cases ...

	TEST CASE			
	MAX _{T1} * WEIGHT =			
	.71	*	0.1	= .07
	.88	*	0.2	= .17
	.97	*	0	= 0
	.87	*	0.2	= .17
				<hr/>
				.41
				FINAL SCORE FOR TEST SEQUENCE#1

Scan a gene (e.g. UGT1A1) for high scoring regions



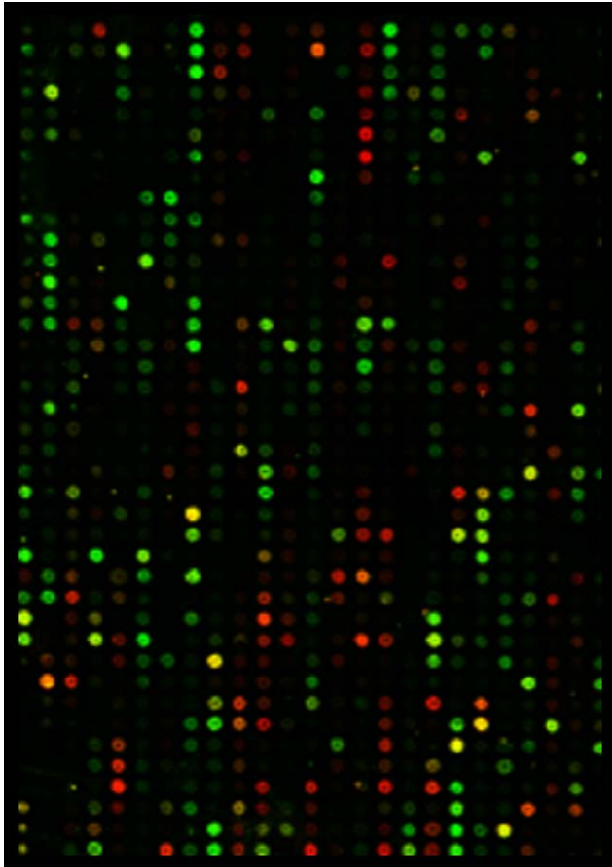
Final Points on CRM Detection

- Most procedures use advanced weighting procedures and do not limit to single maximum scoring TFBS
 - for instance HMMs and Logistic Regression Analysis
- Interpretation of score depends on tolerance for false predictions
 - Most publications assess the false positive rate of CRM prediction procedures at sensitivity of 66%
 - » This point on the sensitivity-specificity spectrum is an artifact of history
- Most trained methods generate false positives at a rate between 1/30000 bp – 1/60000
 - Untrained methods in best cases generate predictions at rates between 1/10000 bp – 1/18000

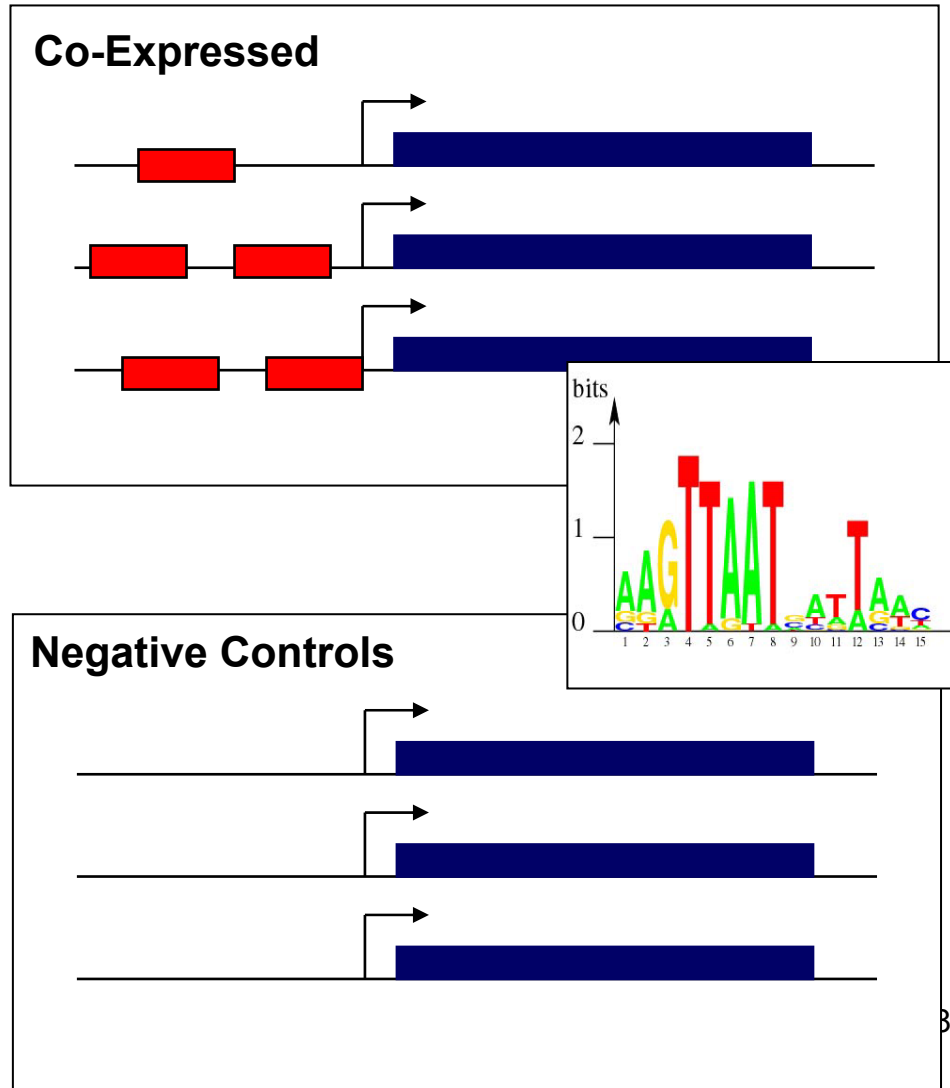
Part 2:

Inferring Regulating TFs for Sets of Co-Expressed Genes

Deciphering Regulation of Co-Expressed Genes



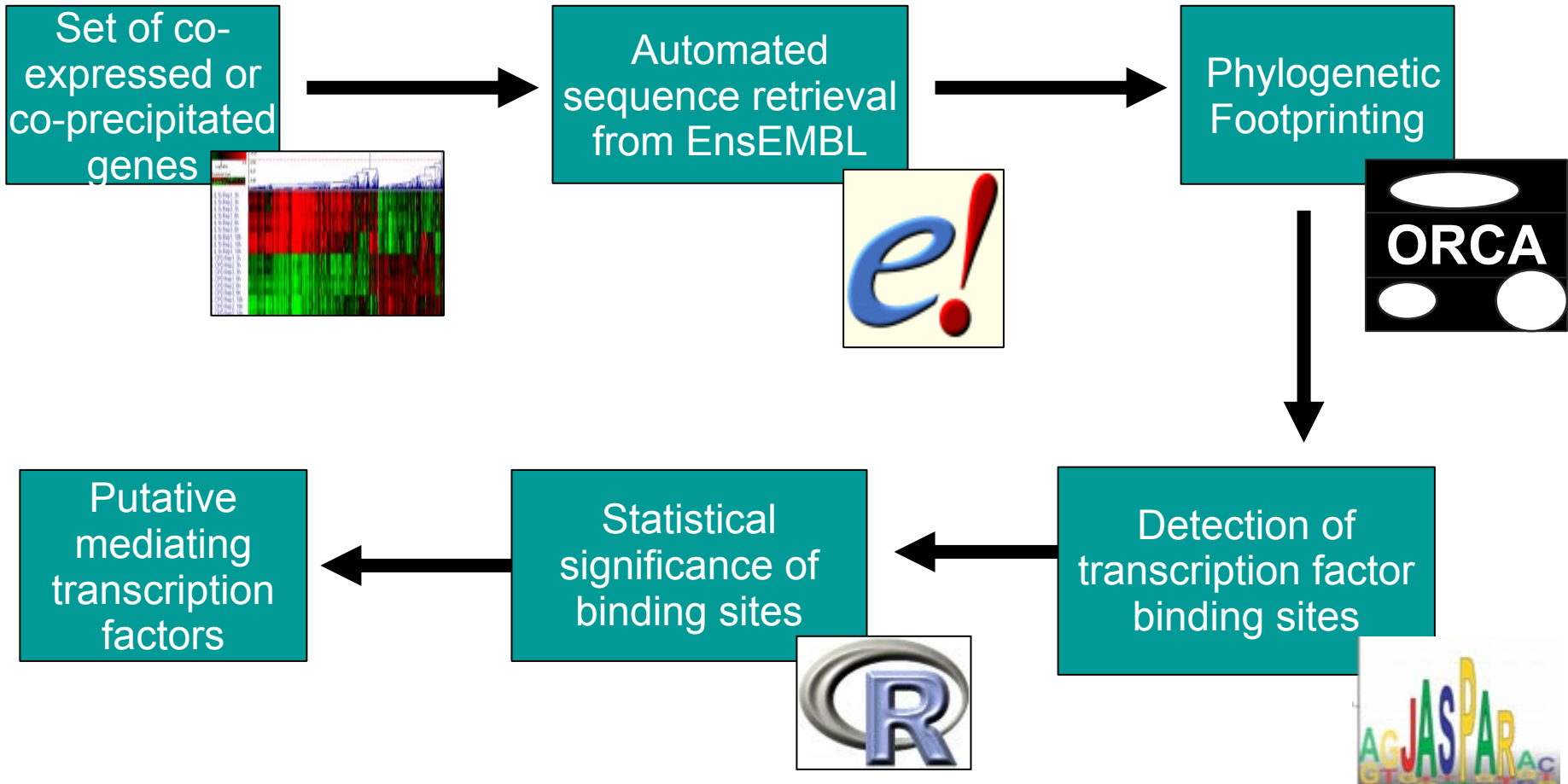
INSERM



TFBS Over-representation

- Akin to the analysis of over-represented GO terms, it would be convenient to identify if a set of co-expressed genes contains an over-abundance of binding sites for a known TF
- We will use phylogenetic footprinting to
- Can over-representation studies be successful?

oPOSSUM Procedure



Statistical Methods for Identifying Over-represented TFBS

- Z scores
 - Based on the **number of occurrences** of the TFBS relative to background
 - Normalized for sequence length
 - Simple binomial distribution model
- Fisher exact probability scores
 - Based on the **number of genes** containing the TFBS relative to background
 - Hypergeometric probability distribution

The oPOSSUM Database

(Not updated for current release)

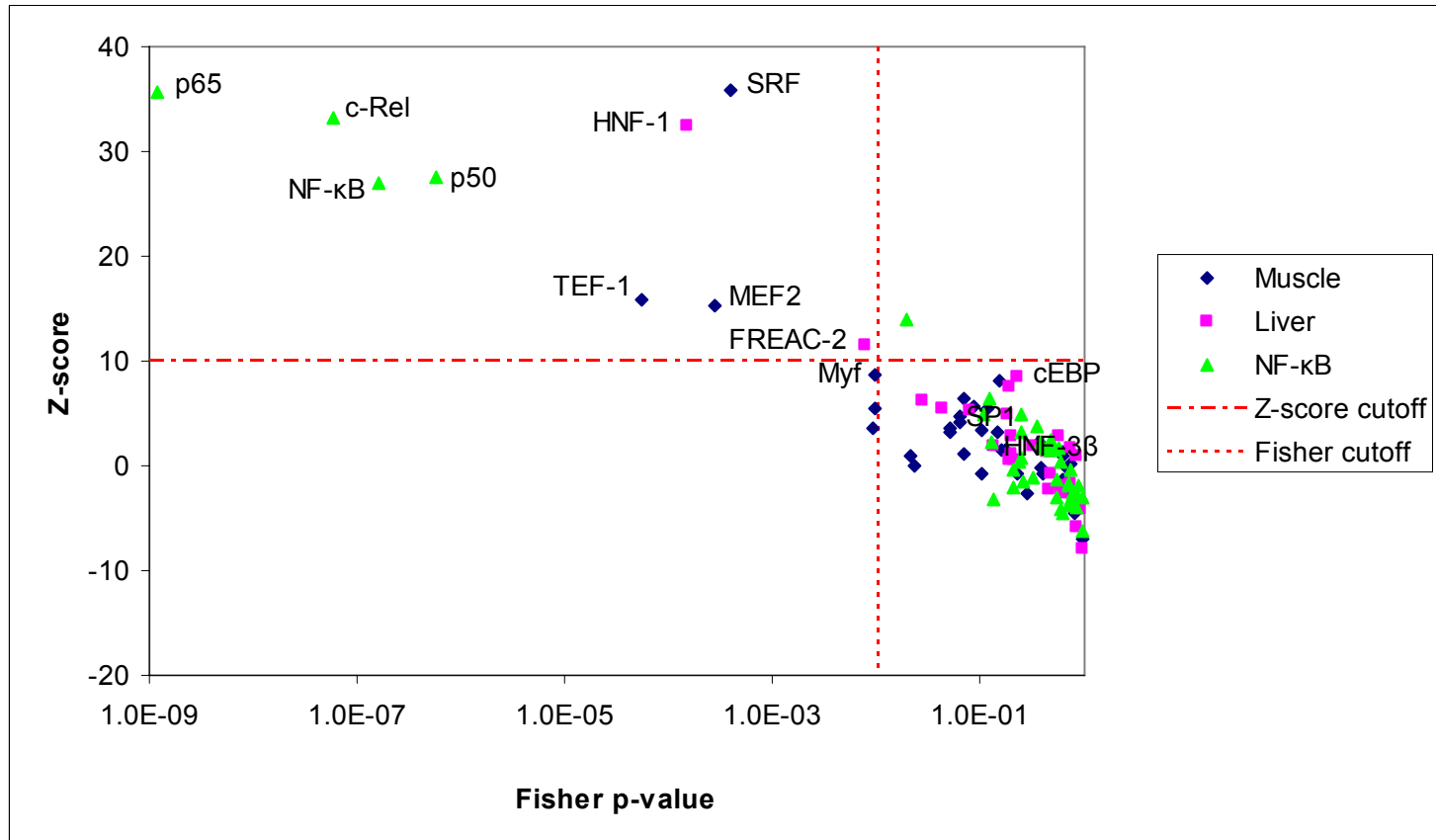
- Orthologous genes: 8468
- Promoter pairs: 6911
- Promoters with TFBS: 6758
- Total # of TFBS predictions: 1638293
- Overall failure rate: 20.2%

Validation using Reference Gene Sets

A. Muscle-specific (23 input; 16 analyzed)				B. Liver-specific (20 input; 12 analyzed)			
	Rank	Z-score	Fisher		Rank	Z-score	Fisher
SRF	← 1	21.41	1.18e-02	HNF-1	← 1	38.21	8.83e-08
MEF2	← 2	18.12	8.05e-04	HLF	2	11.00	9.50e-03
c-MYB_1	3	14.41	1.25e-03	Sox-5	3	9.822	1.22e-01
Myf	← 4	13.54	3.83e-03	FREAC-4	4	7.101	1.60e-01
TEF-1	5	11.22	2.87e-03	HNF-3beta	← 5	4.494	4.66e-02
deltaEF1	6	10.88	1.09e-02	SOX17	6	4.229	4.20e-01
S8	7	5.874	2.93e-01	Yin-Yang	7	4.070	1.16e-01
Irf-1	8	5.245	2.63e-01	S8	8	3.821	1.61e-02
Thing1-E47	9	4.485	4.97e-02	Irf-1	9	3.477	1.69e-01
HNF-1	10	3.353	2.93e-01	COUP-TF	10	3.286	2.97e-01

← TFs with experimentally-verified sites in the reference sets.

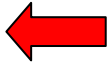

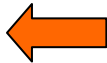
Empirical Selection of Parameters based on Reference Studies



C-Myc SAGE Data

- c-Myc transcription factor dimerizes with the Max protein
- Key regulator of cell proliferation, differentiation and apoptosis
- Menssen and Hermeking identified 216 different SAGE tags corresponding to unique mRNAs that were induced after adenoviral expression of c-Myc in HUVEC cells
- They then went on to confirm the induction of 53 genes using microarray analysis and RT-PCR


Induced Genes after Ectopic Expression of c-Myc (SAGE) (53 input; 36 analyzed)

	TF Class	Rank	Z-score	Fisher	No. Genes
Myc-Max 	bHLH-ZIP	1	21.68	5.35e-03	7
Staf	ZN-FINGER, C2H2	2	20.17	1.70e-02	2
Max 	bHLH-ZIP	3	18.32	2.16e-02	12
SAP-1	ETS	4	13.23	1.61e-04	13
USF	bHLH-ZIP	5	11.90	1.84e-01	16
SP1	ZN-FINGER, C2H2	6	11.68	4.40e-02	12
n-MYC 	bHLH-ZIP	7	11.11	1.55e-01	20
ARNT	bHLH	8	11.11	1.55e-01	20
Elk-1	ETS	9	10.92	3.88e-03	19
Ahr-ARNT	bHLH	10	10.17	1.11e-01	25

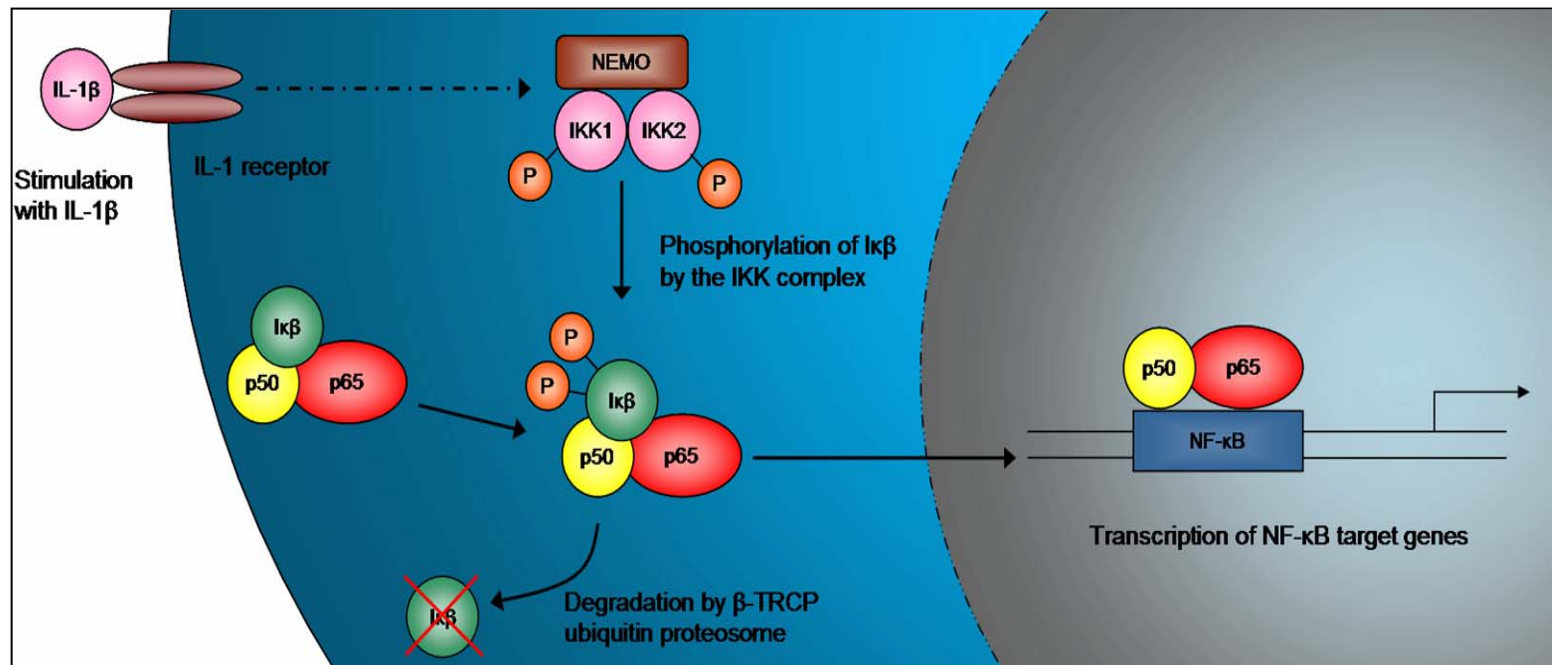
C-Fos Microarray Experiment

- In a study examining the role of transcriptional repression in oncogenesis, Ordway *et al.* compared the gene expression profiles of fibroblasts transformed by c-fos to the parental 208F rat fibroblast cell line
- We mapped the list of 252 induced Affymetrix Rat Genome U34A GeneChip sequences to 136 human orthologs






Induced Genes after Ectopic Expression of c-Fos (Affymetrix) (136 input; 86 analyzed)

	TF Class	Rank	Z-score	Fisher	No. Genes
c-FOS 	bZIP	1	17.53	2.60e-05	45
RREB-1	ZN-FINGER, C2H2	2	8.899	1.41e-01	1
PPARgamma-RXRalpha	NUCLEAR RECEPTOR	3	3.991	2.98e-01	1
CREB	bZIP	4	3.626	1.25e-01	10
E2F	Unknown	5	2.965	7.67e-02	15

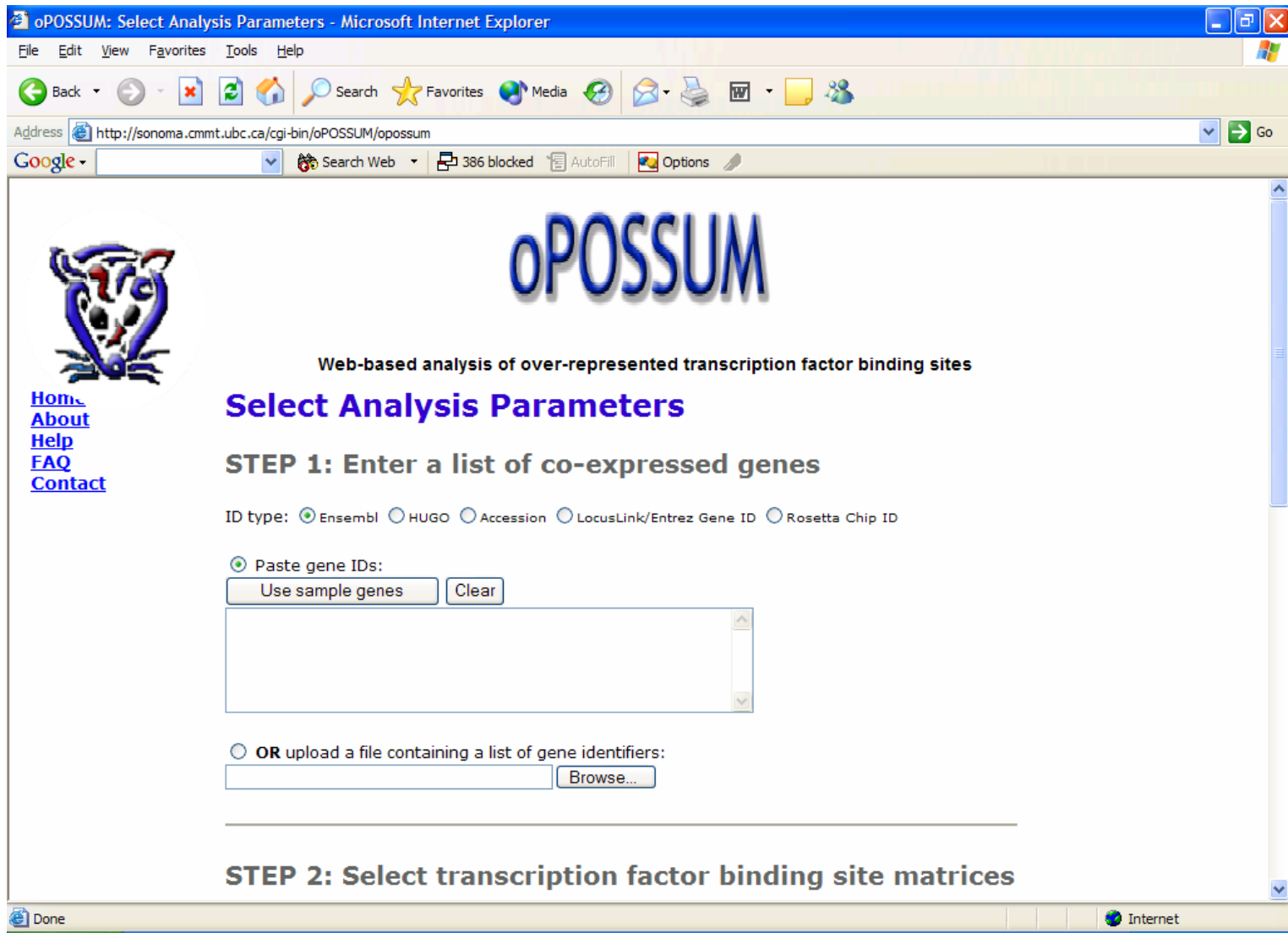
NF- κ B inhibition microarray study



Genes significantly down-regulated by the NF- κ B pathway inhibitor (326 input; 179 analyzed)

		TF Class	Rank	Z-score	Fisher	No. Genes
p65		REL	1	36.57	5.66e-12	62
NF-kappaB		REL	2	32.58	5.82e-11	61
c-REL		REL	3	26.02	8.59e-08	63
Irf-2		TRP-CLUSTER	4	20.39	5.74e-04	6
SPI-B		ETS	5	16.59	1.23e-03	135
Irf-1		TRP-CLUSTER	6	15.4	9.55e-04	23
Sox-5		HMG	7	15.38	2.56e-02	126
p50		REL	8	14.72	2.23e-03	19
Nkx		HOMEODOMAIN	9	13.66	2.29e-03	111
Bsap		PAIRED	10	13.2	9.92e-02	1
FREAC-4		FORKHEAD	11	12.05	1.66e-03	92

oPOSSUM Server



The screenshot shows a web browser window titled "oPOSSUM: Select Analysis Parameters - Microsoft Internet Explorer". The address bar displays "http://sonoma.cmmt.ubc.ca/cgi-bin/oPOSSUM/oportunum". The page features a navigation menu on the left with links for Home, About, Help, FAQ, and Contact. The main content area is titled "oPOSSUM" and "Web-based analysis of over-represented transcription factor binding sites". The current step is "STEP 1: Enter a list of co-expressed genes". Under "ID type", the "Ensembl" radio button is selected. The "Paste gene IDs:" section includes a "Use sample genes" button, a "Clear" button, and a large text input field. The "OR upload a file containing a list of gene identifiers:" section includes a "Browse..." button. The next step is "STEP 2: Select transcription factor binding site matrices". The browser's status bar shows "Done" and "Internet".


oPOSSUM: Select Analysis Parameters - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Back Forward Stop Home Search Favorites Media Refresh Mail Print TV Options

Address <http://sonoma.cmmt.ubc.ca/cgi-bin/oPOSSUM/opossum> Go

Google Search Web 386 blocked AutoFill Options



oPOSSUM

Web-based analysis of over-represented transcription factor binding sites

Select Analysis Parameters

STEP 1: Enter a list of co

ID type: Ensembl HUGO Accession

Paste gene IDs:

Use sample genes Clear

OR upload a file containing a list of gene identifiers:

Browse...

STEP 2: Select transcription factor binding site matrices

Done Internet

INPUT A LIST OF
CO-EXPRESSED GENES

oPOSSUM: Select Analysis Parameters - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Google

Back Forward Stop Refresh Home Search Favorites Media

Address <http://www.cisreg.ca/cgi-bin/oPOSSUM/opossum> Go

STEP 2: Select transcription factor binding site matrices

All profiles with a minimum

OR select by taxonomic s

plant vertebrate inse

OR select specific profiles

- AGL3
- AML-1
- ARNT
- Agamous
- Ahr-ARNT
- Androgen
- Athb-1
- Brachyury

SELECT YOUR TFBS PROFILES

STEP 3: Select parameters

Level of conservation:

Done Internet

oPOSSUM: Select Analysis Parameters - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Google

Back Forward Stop Refresh Home Search Favorites Media Print Mail

Address <http://www.cisreg.ca/cgi-bin/oPOSSUM/opussum> Go

Ahr-ARNT
Androgen
Athb-1
Brachyury

STEP 3: Select parameters

Level of conservation:
1 (top 10.0% of conserved regions)

Matrix match threshold:
80.0 %

Amount of upstream / downstream sequence:
5000 / 5000

Statistical measure for over-representation:
Both

Press the **Submit** button to perform the analysis or **Reset** to reset the analysis parameters to their default values. Depending on server load, the analysis may take anywhere from a few seconds to a minute or more to perform. Please be patient.

SELECT:

1. CONSERVATION
2. PSSM MATCH THRESHOLD
3. PROMOTER REGION
4. STATISTICAL MEASURE

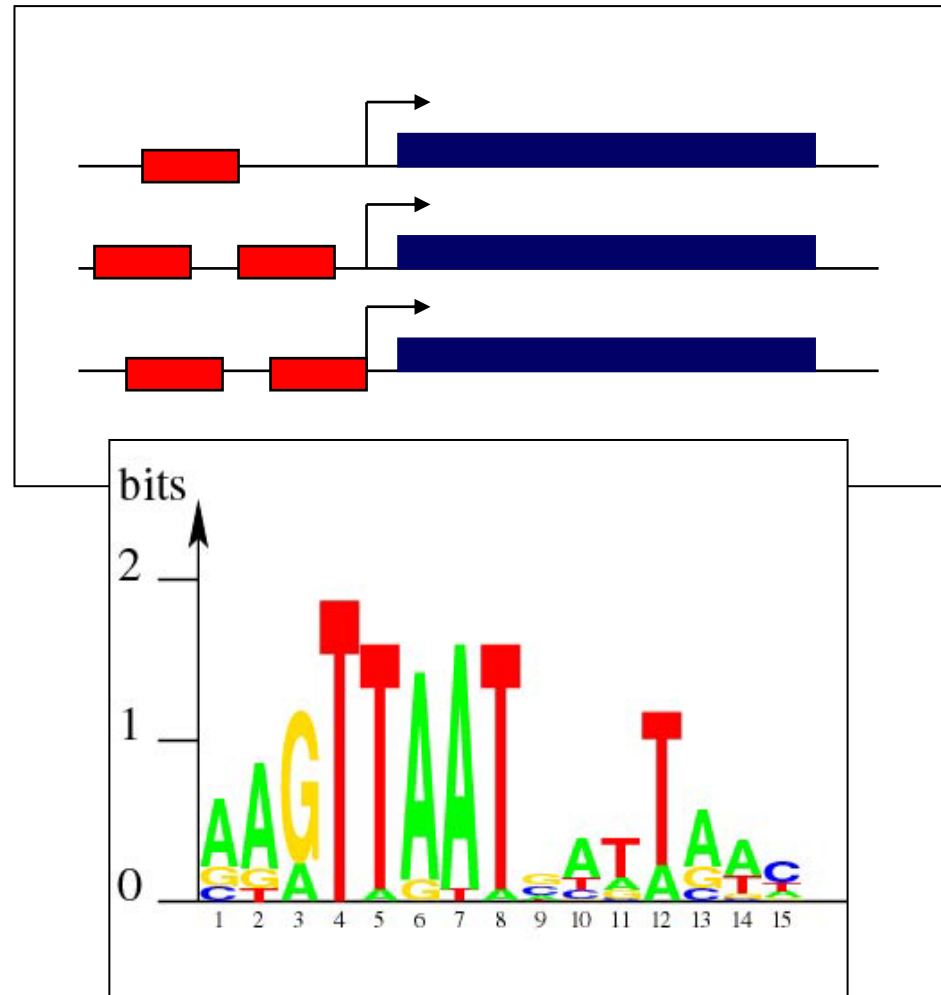
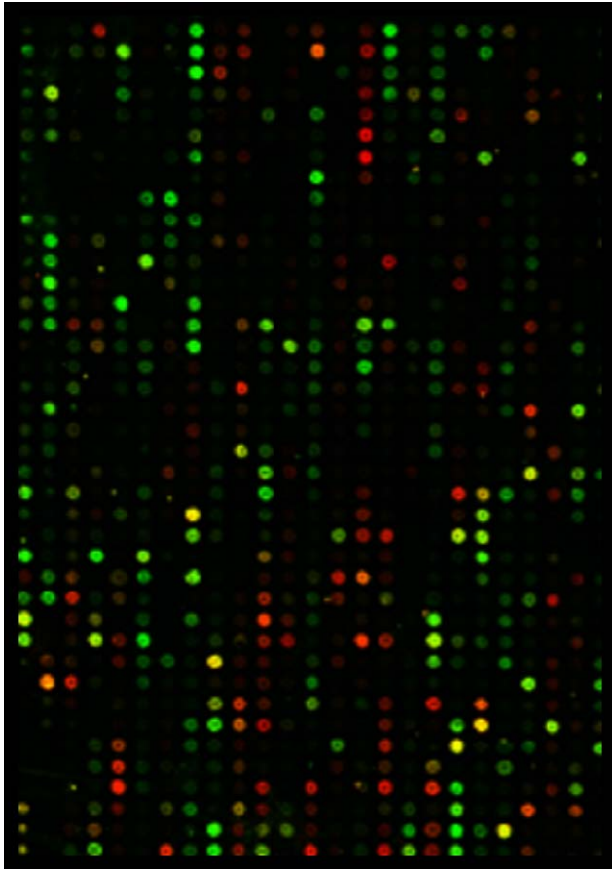
TFBS Over-Representation Summary

- New generation of tools to help interrogate the meaning of observed clusters of co-expressed (hopefully co-regulated) genes
- Convenient API access allows direct queries into the database by informatics staff
- Generally best performance in studies directly linked to a transcription factor
 - Highly dependent on the experimental design – cannot overcome noisy data from poor design
 - ChIP-chip data will be a welcome challenge

Part 3:

de novo Discovery of TF Binding Sites

De novo Pattern Discovery



de novo Pattern Discovery

- String-based
 - e.g. YMF (Sinha & Tompa)
 - Generalization: Identify over-represented oligomers in comparison of “+” and “-” (or complete) promoter collections
 - Used often for yeast promoter analysis
- Profile-based
 - e.g. Motif Sampler (Lawrence) or MEME (Bailey & Elkin)
 - Generalization: Identify strong patterns in “+” promoter collection vs. background model of expected sequence characteristics

String-based methods(1)

How likely are X words in a set of sequences, given background sequence characteristics?

```
CCCCCCGGAATGAAATCTGATTGACATTTTCC >EP71002 (+) Ce[IV] msp-56 B; range -100 to -75
TTCAAATTTTAAACGCCGGAATAATCTCCTATT >EP63009 (+) Ce Cuticle Col-12; range -100 to -75
TCGCTGTAACCGGAATATTTAGTCAGTTTTTG >EP63010 (+) Ce Cuticle Col-13; range -100 to -75
TATCGTCATTCTCCGCCTCTTTTCTT >EP11013 (+) Ce vitellogenin 2; range -100 to -75
GCTTATCAATGCGCCCGGAATAAAACGCTATA >EP11014 (+) Ce vitellogenin 5; range -100 to -75
CATTGACTTTATCGAATAAATCTGTT >EP11015 (-) Ce vitellogenin 4; range -100 to -75
ATCTATTTACAATGATAAACTTCAA >EP11016 (+) Ce vitellogenin 6; range -100 to -75
ATGGTCTCTACCGGAAAGCTACTTTTCAGAATT >EP11017 (+) Ce calmodulin cal-2; range -100 to -75
TTTCAAATCCGGAATTTCCACCCGGAATTACT >EP63007 (-) Ce cAMP-dep. PKR P1+; range -100 to -75
TTTCCTTCTTCCGGAATCCACTTTTCTTCC >EP63008 (+) Ce cAMP-dep. PKR P2; range -100 to -75
ACTGAACTTGTCTTCAAATTTCAACACCGGAA >EP17012 (+) Ce hsp 16K-1 A; range -100 to -75
TCAATGCCGGAATTCTGAATGTGAGTCGCCCT >EP55011 (-) Ce hsp 16K-1 B; range
```

String-based methods(2)

Find all words of length n in the yeast promoters (e.g. $n=7$)

```
GTCTTATCTTCAAAGTTGTCTGTCCAAGATTTGGACTTGAAGG
ACAAGCGTGTCTTCTCAGAGTTGACTTCAACGTCCCATTGGAC
GGTAAGAAGATCACTTCTAACCAAAGAATTGTTGCTGCTTTGC
CAACCATCAAGTACGTTTTGGAACACCACCAAGATACGTTGT
CTTGTTCTCACTTGGGTAGACCAAACGGTCAAAGAAAACGAAAA
ATACTCTTTGGCTCCAGTTGCTAAGGAATTGCAATCATTGTTG
GGTAAGGATGTCACCTTCTTGAACGACTGTGTCGGTCCAGAA
GTTGAAGCCGCTGTCAAGGCTTCTGCCCCAGGTTCCGTTATTT
TGTTGGAAAACGCGTTACCACATCGAAGAAGAAGGTTCCAGA
AAGGTCGATGGTCAAAAAGGTC AAGGCTCAAGGAAGATGTTCA
AAAGTTCAGACACGAATTGAGCTCTTTGGCTGATGTTTACATC
ACGATGCCTTCGGTACCGCTCACAGAGCTCACTCTTCTATGGT
CGGTTTCGACTTGCCAACGTGCTGCCGGTTTCTTGTTGGAAAA
GGAATTGAAGTACTTCGGTAAGGCTTTGGAGAACCCAACCAG
ACCATTCTTGCCATCTTAGGTGGTGCCAAGGTTGCTGACAAG
ATTCAATTGATTGACAACCTTGGTGGACAAGGTCGACTCTATCAT
CATTGGTGGTGGTATGGCTTTCCTTCAAGAAGGTTTTGGAAA
ACACTGAAATCGGTGACTCCATCTTCGACAAGGCTGGTGCTG
AAATCGTTCCAAAGTTGATGGAAAAGGCCAAGGCCAAGGGTG
TCGAAGTCGTCTTGCAAGTCACTTCACTGACTGACTGACTGCTTTC
TCTGCTGATGCCAACACCAAGACTGTCACTGACAAGGAAGGT
ATTCCAGCTGGCTGGCAAGGTTGGACAATGGTCCAGAATCT
AGAAAAGTGTGTTGCTGCTACTGTTGCAAAGGCTAAGACCATTGT
CTGGAACGGTCCACCAGGTGTTTTCGAATTCGAAAAGTTCGCT
GCTGGTACTAAGGCTTTGTTAGACGAAGTTGTCAAGAGCTCTG
CTGCTGGTAACACCGTCATCATTGGTGGTGGTGACTGCCA
```

INSERM

Make a lookup table:

AAAAAAA	57788
AAACCTT	456
GATAGCA	589

Etc...



String-based methods(3)

$$Z_w = \frac{X_w - E[X_w]}{\text{Var}[X_w]}$$

X_w : Instances of a word w within our set of X genes

$E[X_w]$: Average number of instances of w based on number of genes in our set

$\text{Var}[X_w]$: Variance – how much deviation from the average is expected for w

String-based methods(4)

STRING	Total (promoters)	Observed	Z
AAAAAAAA	5788	140	2
.			
.			
.			
AAACCTT	456	125	21
.			
.			
.			
GATAGCA	589	16	1
.			
.			
.			

Limitations of String-based Methods

- Longer word lengths not computationally practical
- While many methods use degeneracy codes, TFBS are not words – dilutes the signal we are seeking
 - Imagine a "true" pattern represented at a position with 7 A's and 1 T...
 - We throw out the instance with T...
 - Now imagine next position with 6 C's and 1 G...

Probabilistic Methods for Pattern Discovery

- What is a probabilistic method?
- The Gibbs sampler algorithm

Probabilistic Methods

Overview:

Find a local alignment of width x of sites that **maximizes a scoring function** (commonly MAP score) in reasonable time

Usually by Gibbs sampling or EM methods

Motivation:

TFBS are not words

Efficiency – can handle longer patterns than string-based methods

Can be intentionally influenced to reflect prior knowledge

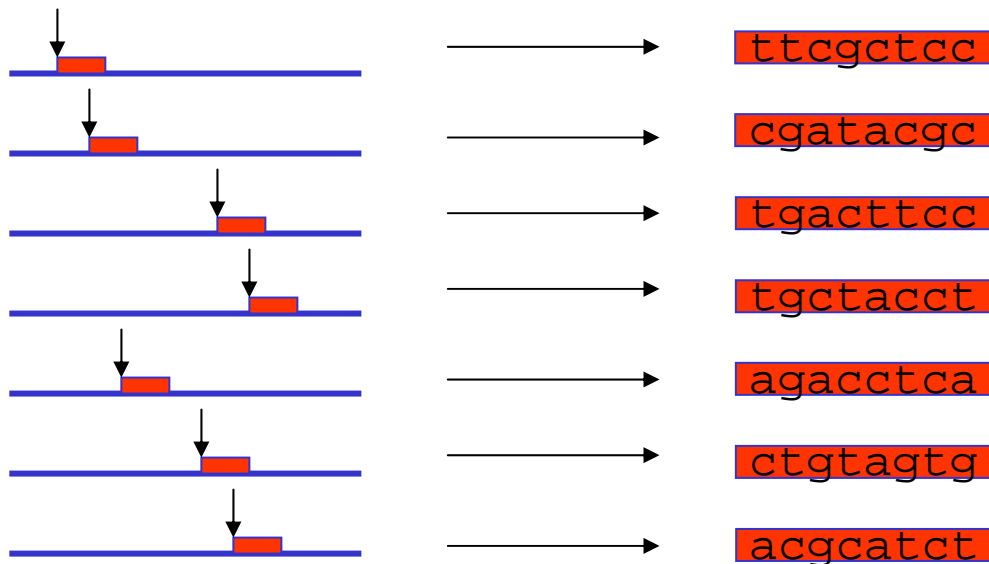
What does probabilistic mean?

- Based on probability
- Functionally, it means we're going to guess our way to a good pattern (TFBS)
 - We're going to try to make a good guess
- Two different flavours of the approach
 - Expectation Maximization in which we make our best guess each time
 - Gibbs Sampling in which we make our guesses based on the strength of our conviction (our best guess is usually only slightly better than our second best guess)

Gibbs Sampling (1)

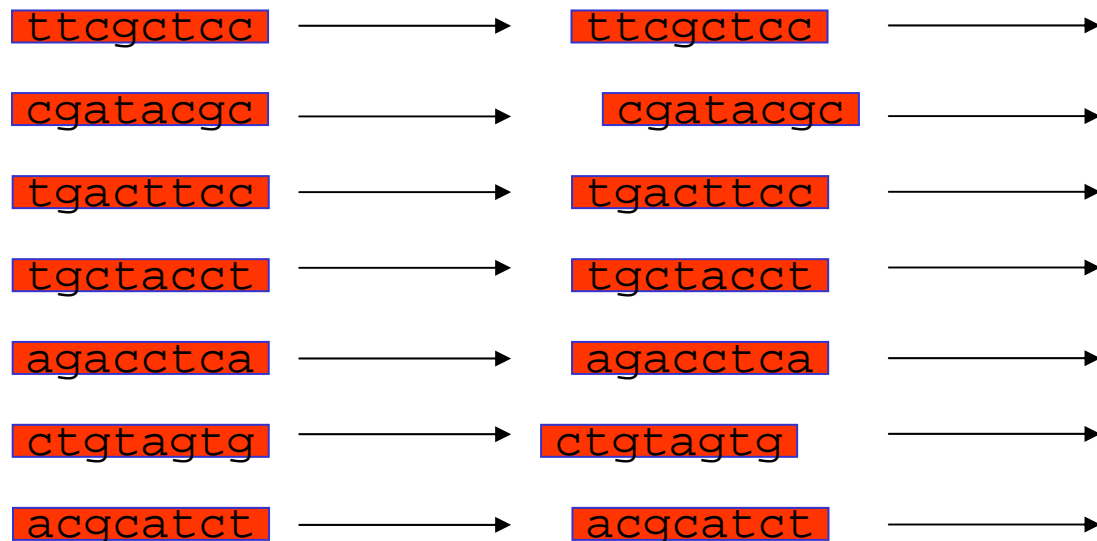
(grossly over-simplified)

Guess the positions of the binding sites (user often selects number of occurrences and the length of the motif to be found)



Gibbs Sampling (2)

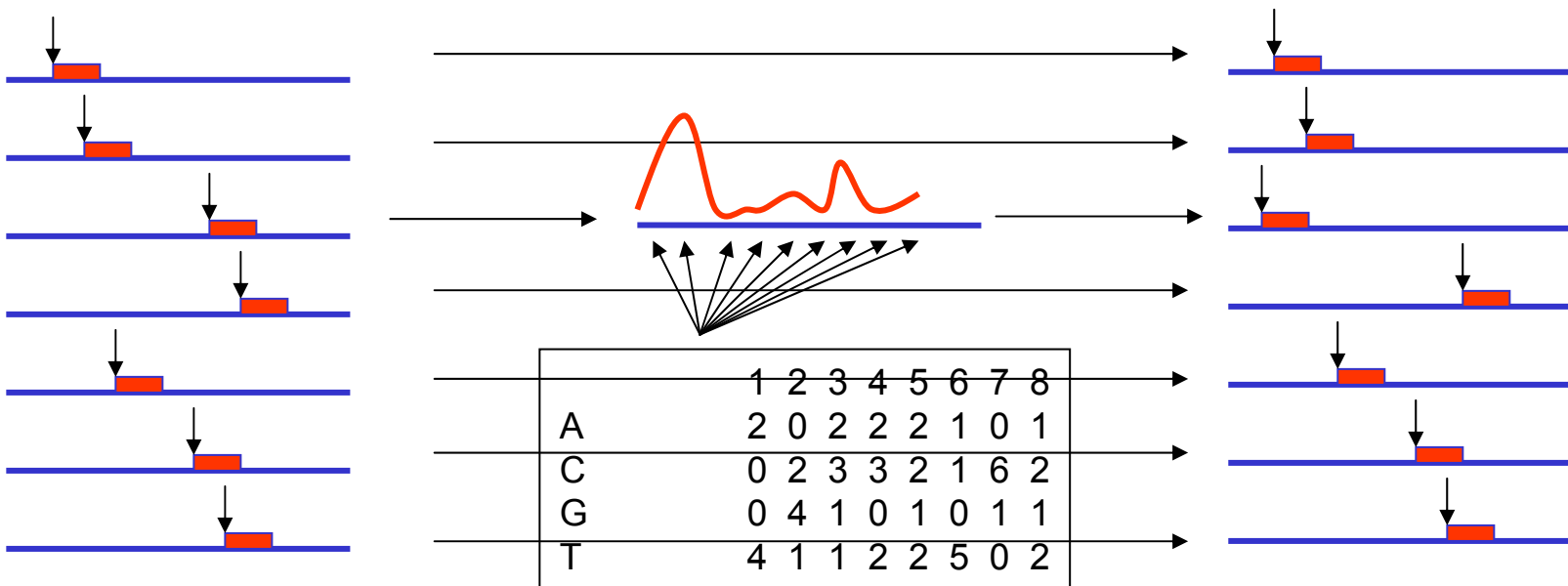
Align the sites and construct a scoring matrix...



	1	2	3	4	5	6	7	8
A	2	0	2	2	2	1	0	1
C	0	2	3	3	2	1	6	2
G	0	4	1	0	1	0	1	1
T	4	1	1	2	2	5	0	2

Gibbs Sampling (3)

For one of your sequences, throw out the site and guess a new site based on the TFBS scores generated with your matrix...
Return to Step #2 (align sites)

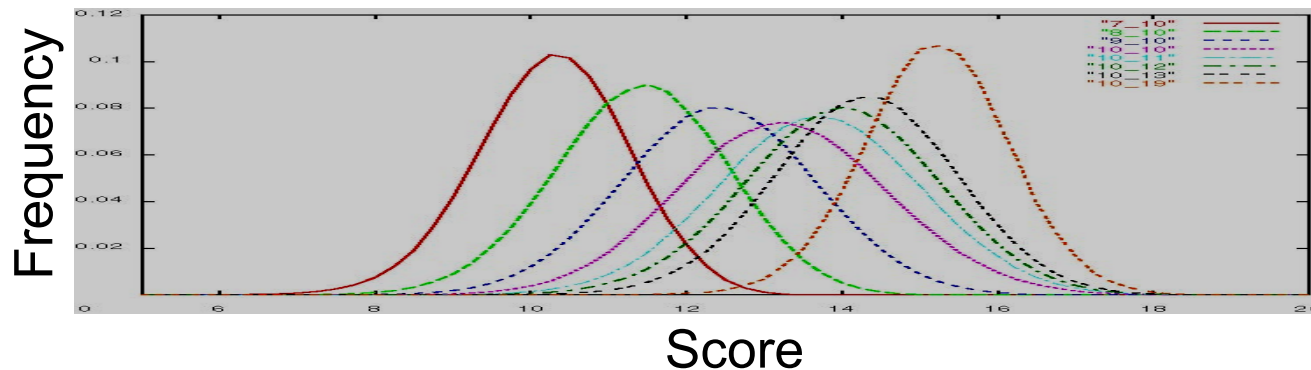


How to assess the quality of the pattern returned?

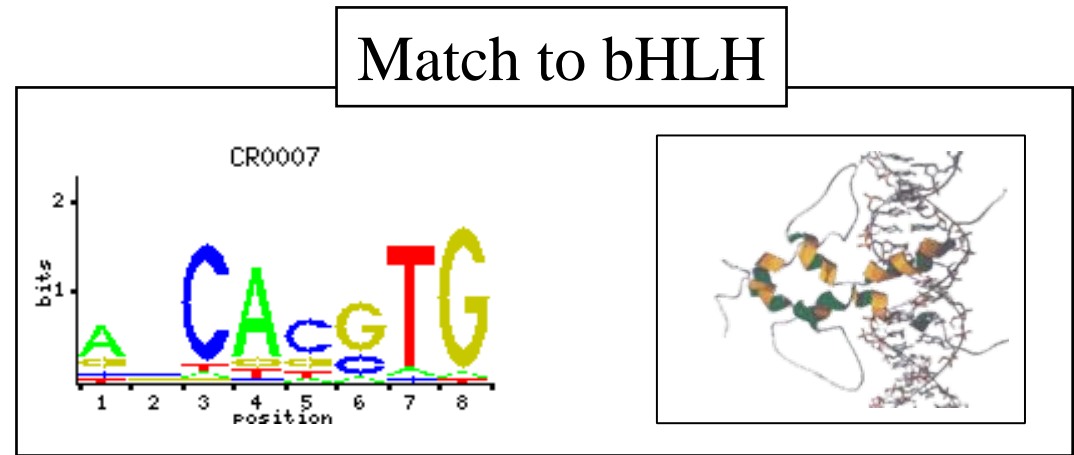
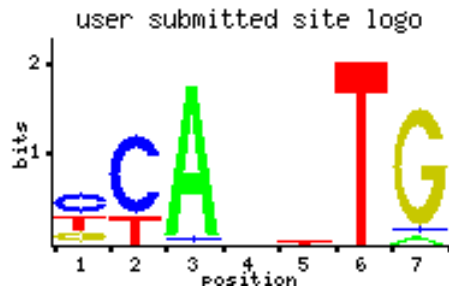
- How would you assess the relevance of a cDNA sequence that you cloned?
 - BLAST IT!!!!!!!!!!
- How can we compare our pattern to a database of patterns...?

Comparison of profiles requires alignment and a scoring function

- Scoring function based on sum of squared differences
- Align frequency matrices with modified Needleman-Wunsch algorithm
- Calculate empirical p-values based on simulated set of matrices



Intra-family comparisons more similar than inter-family



Jackknife Test 87% correct

Independent Test Set 93% correct

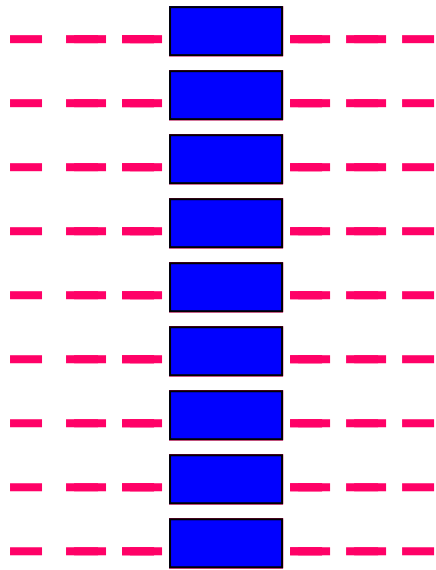
How to assess the quality of the pattern returned?

- How would you assess the relevance of a cDNA sequence that you cloned? First step? BLAST?
 - Compare our pattern to a database of patterns
- (Not shown) We could determine if our pattern is present in the same set of genes in other species (preferably excluding the genes used to build the pattern)
 - I call this procedure Regulog analysis - excluded for time

Pattern Discovery

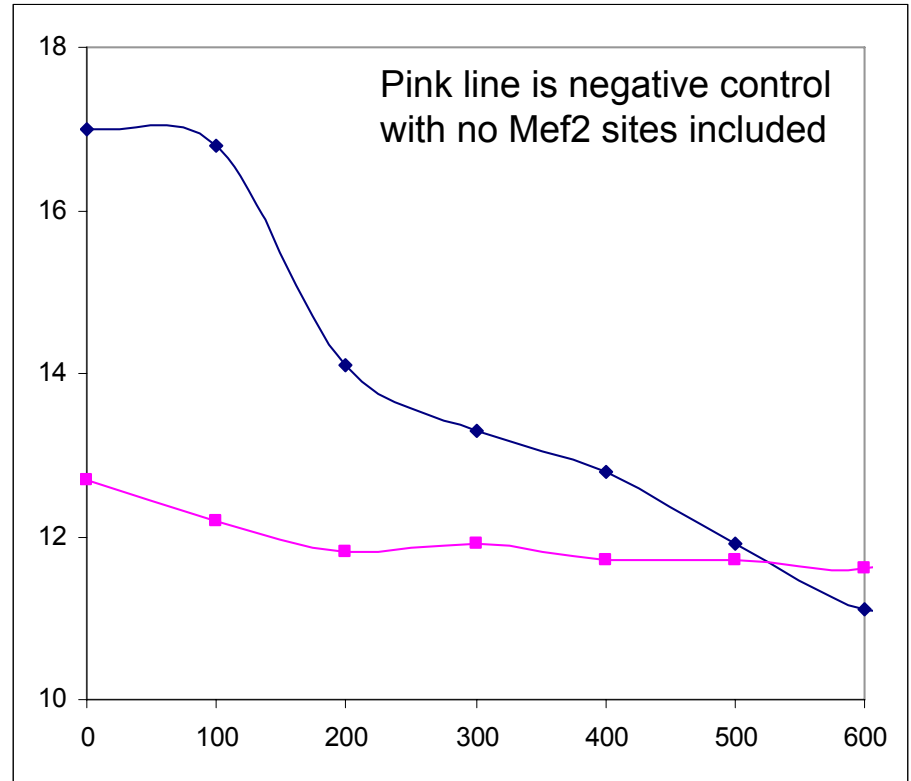
- Gibbs sampling can get stuck on less than optimal patterns depending on initialization conditions
 - Procedure is fast, so running many 1000s of times is feasible
- Unfortunately...what if our pattern of interest is not strong relative to irrelevant patterns...

Applied Pattern Discovery is Acutely Sensitive to Noise



True Mef2 Binding Sites

PATTERN SIMILARITY
VS. TRUE MEF2 PROFILE



SEQUENCE LENGTH

Some Approaches to Improve Sensitivity

- Better background models (changes the preferences for guessing)
 - Higher-order properties of DNA
- Phylogenetic Footprinting (changes the preferences for guessing)
 - Human:Mouse comparison eliminates ~75% of sequence
- Regulatory Modules (changes the scoring function)
 - Architectural rules
- Limit the types of binding profiles allowed
 - TFBS patterns are NOT random

Pattern Discovery Summary

- Pattern discovery methods can recover over-represented patterns in the promoters of co-expressed genes
- Methods are acutely sensitive to noise, indicating that the signal we seek is weak
 - TFs tolerate great variability between binding sites
- As for pattern discrimination, supplementary information/approaches are required to overcome the noise
- Except in yeast, not quite ready for real world problems

REFLECTIONS

- Part 1
 - Futility Conjecture – Essentially predictions of individual TFBS have no relationship to an *in vivo* function
 - Successful bioinformatics methods for site discrimination incorporate additional information (clusters, conservation)
- Part 2
 - TFBS over-representation is a power new means to identify TFs likely to contribute to observed patterns of co-expression
- Part 3
 - Pattern discovery methods are severely restricted by the Signal-to-Noise problem
 - Observed patterns must be carefully considered
 - Successful methods for pattern discovery will have to incorporate additional information (conservation, structural constraints on TFs)

Thank you for listening...

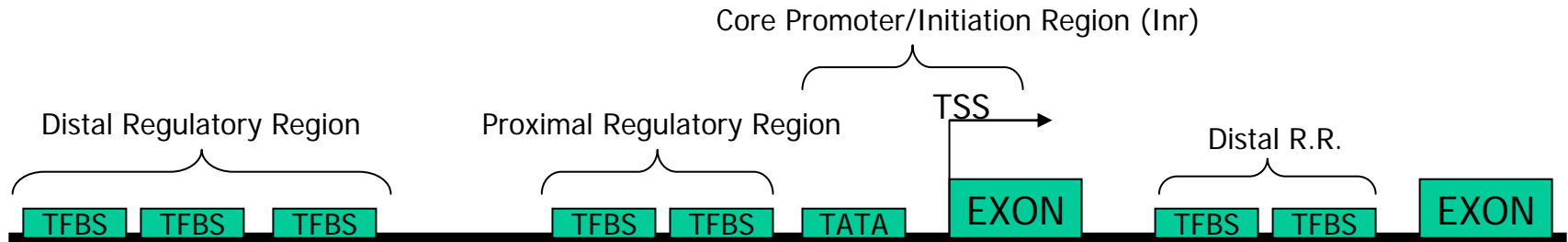
- ConSite
 - Boris Lenhard (U.Bergen), Albin Sandelin (RIKEN), Luis Mendoza (Serono)
- oPOSSUM
 - Shannan Ho Sui (UBC), Dave Arenillas (UBC), James Mortimer (Merck)
- Matrix Comparison
 - Albin Sandelin
- Regulog Analysis
 - Wynand Alkema (Organon)
- JASPAR
 - Albin Sandelin, Boris Lenhard
 - Watch for the new JASPAR coming soon (Elodie Portales-Casamar(UBC) and Stefan Kirov (Oak Ridge))

THE END

Questions?

Anatomy of Transcriptional Regulation

WARNING: Terms vary widely in meaning between scientists



- Core Promoter – Sufficient to support the initiation of transcription; orientation dependent
 - TSS – transcription start site
 - Often a region rather than specific position
 - Often multiple in same gene
- TFBS – single transcription factor binding site
- Regulatory Regions
 - Proximal/Distal – vague reference to distance from TSS
 - May be positive (enhancing) or negative (repressing)
 - Orientation independent (generally)
 - Modules – Sets of TFBS within a region that function together