Regulatory Sequence Analysis

Pattern discovery
String-based approaches

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Detection of over-represented patterns

- Knowing that a set of genes are co-regulated, one can expect that their upstream regions contains some regulatory signal.
- This signal is likely to be more frequent in the upstream regions of the co-regulated genes than in a random selection of genes.
- In order to discover signals responsible for the co-regulation of a group of genes, we will thus detect over-represented patterns in their upstream sequences.

Evaluation with known regulons

Testing the performances with known regulons

- NIT
  - 7 genes expressed under low nitrogen conditions
- MET
  - 10 genes expressed in absence of methionine
- PHO
  - 5 genes expressed under phosphate stress
- GAL
  - 6 genes expressed in presence of galactose
- ...

Interface between the yeast Pho4p protein and one of its binding sites

Background model

- In order to detect over-represented patterns, the observed occurrences are compared to the random expectation.
- The random expectation can be estimated according to different models
  - Bernouilli model, with a specific probability for each nucleotide.
  - Markov model, calibrated on the basis of the input sequence itself.
  - External background : occurrences for the same pattern in a reference data set
    - whole genome
    - intergenic sequences
    - set of all upstream sequences for the organism considered

The most frequent oligonucleotides are not informative

- A (too) simple approach would consist in detecting the most frequent oligonucleotides (for example hexanucleotides) for each group of upstream sequences.
- This would however lead to deceiving results.
  - In all the sequence sets, the same kind of patterns are selected: AT-rich hexanucleotides.

PHO

aaaaaa|tttttt	51
aaaaag|cttttt	15
aagaaa|tttctt	14
gaaaaa|tttttc	13
tgccaa|ttggca	12
aaaaat|attttt	12
aaatta|taattt	12
agaaaa|ttttct	11
caagaa|ttcttg	11
aaacgt|acgttt	11
aaagaa|ttcttt	11
acgtgc|gcacgt	10
aataat|attatt	10
aagaag|cttctt	10
atataa|ttatat	10

MET

aaaaaa|tttttt	105
atatat|atatat	41
gaaaaa|tttttc	40
tatata|tatata	40
aaaaat|attttt	35
aagaaa|tttctt	29
agaaaa|ttttct	28
aaaata|tatttt	26
aaaaag|cttttt	25
agaaat|atttct	24
aaataa|ttattt	22
taaaaa|ttttta	21
tgaaaa|ttttca	21
ataata|tattat	20
atataa|ttatat	20

NIT

aaaaaa|tttttt	80
cttatc|gataag	26
tatata|tatata	22
ataaga|tcttat	20
aagaaa|tttctt	20
gaaaaa|tttttc	19
atatat|atatat	19
agataa|ttatct	17
agaaaa|ttttct	17
aaagaa|ttcttt	16
aaaaca|tgtttt	16
aaaaag|cttttt	15
agaaga|tcttct	14
tgataa|ttatca	14
atataa|ttatat	14

GAL

aaaaaa|tttttt	47
aaaaat|attttt	17
aatata|tatatt	17
aaaatt|aatttt	16
aaaata|tatttt	15
attttc|gaaaat	13
aaataa|ttattt	13
aaatat|atattt	13
ataaaa|ttttat	12
atatta|taatat	12
atatat|atatat	11
tgaaaa|ttttca	11
caaaaa|tttttg	11
taaaaa|ttttta	11
agatat|atatct	11

A more relevant criterion for over-representation

- A more relevant criterion for over-representation is to detect patterns which are more frequent in the upstream sequences of the selected genes (co-regulated) than the random expectation.
- The random expectation is calculated by counting the frequency of each pattern in the complete set of upstream sequences (all genes of the genome).

Estimation of word-specific expected frequencies with a Markov chain model trained on the input sequences

$$P(S,m) = P(S_{1,m}) \prod_{i=m+1}^{L} P\left(r_i \mid S_{i-m,i-1}\right)$$

The probability to find a letter at position *i* depends on the residues found at the *m* preceding residues. The tables represent the transition matrices for Markov chain models of order 1 (top) and 2 (bottom). Expected frequencies are estimated from the whole set of yeast upstream sequences.

Transition matrix, order 1

	g	a	c	t
a	0.178	0.369	0.165	0.288
c	0.166	0.327	0.191	0.316
g	0.190	0.313	0.211	0.286
t	0.175	0.273	0.180	0.372

Transition matrix, order 2

	g	a	c	t
aa	0.185	0.411	0.152	0.252
ac	0.171	0.348	0.186	0.296
ag	0.193	0.337	0.201	0.269
at	0.163	0.343	0.167	0.326
ca	0.181	0.344	0.184	0.291
cc	0.168	0.313	0.198	0.321
cg	0.194	0.283	0.227	0.295
ct	0.187	0.240	0.189	0.384
ga	0.186	0.407	0.145	0.262
gc	0.180	0.331	0.194	0.295
gg	0.192	0.318	0.216	0.274
gt	0.199	0.305	0.159	0.338
ta	0.160	0.304	0.182	0.354
tc	0.151	0.313	0.192	0.344
tg	0.184	0.302	0.210	0.304
tt	0.168	0.220	0.195	0.417

Estimation of word-specific expected frequencies from a set of background sequences

Example:
6nt frequencies in the whole set of yeast upstream sequences
Words are grouped by pairs of reverse complements.

;seq	identifier	observed_freq	occ
aaaaaa	aaaaaa|ttttt	0.00510699	14555
aaaaac	aaaaac|gtttt	0.00207402	5911
aaaaag	aaaaag|ctttt	0.00375191	10693
aaaaat	aaaaat|atttt	0.00423577	12072
aaaaca	aaaaca|tgttt	0.0019828	5651
aaaacc	aaaacc|ggttt	0.00088526	2523
aaaacg	aaaacg|cgttt	0.00090105	2568
aaaact	aaaact|agttt	0.0014621	4167
aaaaga	aaaaga|tcttt	0.00323016	9206
aaaagc	aaaagc|gcttt	0.00135824	3871
aaaagg	aaaagg|ccttt	0.0017849	5087
aaaagt	aaaagt|acttt	0.0019035	5425
aaaata	aaaata|tattt	0.00336805	9599
aaaatc	aaaatc|gattt	0.00131368	3744
aaaatg	aaaatg|cattt	0.00185648	5291
aaaatt	aaaatt|aattt	0.00269156	7671
aaacaa	aaacaa|ttgtt	0.00209999	5985
aaacac	aaacac|gtgtt	0.00071684	2043
aaacag	aaacag|ctgtt	0.00096491	2750
aaacat	aaacat|atgtt	0.00108982	3106
aaacca	aaacca|tggtt	0.00074421	2121

- Hexanucleotide frequencies of have been measured in the whole set of 6000 yeast upstream sequences.
- Some words are very frequent, others are rare.
  - range 4.5e-5 to 1.2e-2
  - max(f)/min(f)=268

Hexanucleotide frequencies in yeast upstream sequences

6nt frequencies differ between coding and non-coding sequences

Inter-species variations in intergenic 6nt frequencies

Hexanucleotide occurrences
in upsteam sequences of the NIT family
observed occurrences
expected occurrences
0
10
20
30
40
50
60
70
80
90
AAAAAA
TTTTTT
TATATA
ATATAT
GATAAG
ATAAGA
AAATTT

Hexanucleotide occurrences
in upsteam sequences of the MET family
observed occurrences
expected occurrences
0
10
20
30
40
50
60
70
80
90
100
110
AAAAAA
TTTTTT
ATATAT
TATATA
CACGTG
ACGTGA

Hexanucleotide occurrences
in upsteam sequences of the PHO family
observed occurrences
expected occurrences
0
10
20
30
40
50
60
AAAAAA
TTTTTT
TATATA
CACGTG
AATATT

Hexanucleotide occurrences
in upsteam sequences of the GAL family
AAAAAA
TTTTTT
observed occurrences
expected occurrences
0
10
20
30
40
50
60

Hexanucleotide occurrences in yeast NIT, PHO, MET and GAL upstream sequences

Hexanucleotide occurrences in an extended NIT family

- We analyzed here an extended set of 41 NIT genes (taken from Godard et al., 2006).
- The number of genes affects the dispersion around the diagonal on the plot of observed versus expected occurrences.
- The signal-to-noise separation increases when more genes are analyzed.
- The logarithmic axes better emphasize the words with low expected and observed occurrences but does not allow to display words with 0 occurrences.
- Words with very low expected frequencies are sensitive to low-number fluctuations. For such cases, the observed/expected ratio is misleading (e.g. exp=1, obs=4).

Scoring statistics

- Several scoring statistics have been used to assess the statistical significance of word over-representation
- Observed/expected ratio
  - Never use this statistics !
  - The ratio can be misleading, because it over-emphasizes the patterns with a very low number of expected number of occurrences
  - Example:
    - x_{obs}/x_{exp} = 10/1 is quite significant, but x_{obs}/x_{exp} =1/0.1 is not.
- Log-likelihood ratio
  - $LLR=F_{obs}*log(F_{obs}/F_{exp})$
- Z-score (Matthieu Blanchette)
  - $Z\text{-}score = (x_{obs}-x_{exp})/s_X$
  - Only valid for very large sequences (exp >> 10 for each word)
- Poisson (Andreas Wagner)
- Compound Poisson (Sophie Schbath)
- Binomial (Jacques van Helden)

Scoring scheme - Binomial

- Advantages
  - Allows to estimate a P-value.
  - Appropriate for small sequence sets, where some words have a very low expected number of occurrences (<1).
  - Allows to detect over- and under-representation.
- Weaknesses
  - Bias for self-overlapping words (but this can be circumvented by preventing the counting of overlapping occurrences).
  - Assumes that sequence length is much larger than word length

- Probability to observe exactly x occurrences

$$P(X = x) = \frac{T!}{x!(T-x)!} p^x (1-p)^{T-x}$$

- Probability to observe at least s occurrences

$$P(X \geq x) = \sum_{i=x}^{T} \frac{T!}{i!(T-i)!} p^i (1-p)^{T-i}$$

Where

x = observed occurrences

$T = Sum_{i=1->n}(L_i\text{-}k+1)$ = number of possible positions for a word of length k in a sequence of n sequences of length L_i

p = word probability

Hexanucleotide analysis of the NIT family

Sequence	exp freq	occ	exp occ	P-value	E-value	sig	matching sequences
...ATAAGa	0.00110	18	6.1	6.20E-05	1.30E-01	0.89	6
..GATAAG.	**0.00053**	**24**	**2.9**	**1.20E-14**	**2.60E-11**	**10.59**	**6**
.cGATAA..	0.00048	10	2.7	0.00044	9.20E-01	0.04	5
ctGATA...	0.00052	11	2.9	0.00019	4.00E-01	0.4	6
acatct	0.00051	11	2.8	0.00016	3.40E-01	0.47	4

Genes	*DAL5, DAL80, GAP1, MEP1, MEP2, MEP3, PUT4*
Known motifs	*Factors*
GATAAg	*Gln3p; Nil1p; Gzf3p; Uga43p*

Feature-map of discovered patterns - NIT family

- Typical features of yeast GATA-boxes
  - Multiple occurrences per sequences.
  - Occurrences generally appear clustered (at least two with a spacing of 0-60bp).
  - This probably stimulates synergic effects.
- Remark: PUT4 does not contain a single optimal motif

Hexanucleotide analysis of the PHO family

Sequence	exp freq	occ	exp occ	P-value	E-value	sig	matching sequences
.....CGTGGG	0.00013	5	0.5	0.00021	4.30E-01	0.36	3
....ACGTGc.	**0.00021**	**9**	**0.8**	**2.50E-07**	**5.20E-04**	**3.29**	**5**
....ACGTGG.	0.00018	7	0.7	9.00E-06	1.90E-02	1.73	5
...CACGTG..	0.00012	6	0.5	8.90E-06	1.90E-02	1.73	5
.cgCACG....	0.00013	6	0.5	1.40E-05	2.90E-02	1.54	5
ctgCAC...	0.00024	8	1.0	7.80E-06	1.60E-02	1.79	4
....ACGTTT.	0.00061	10	2.4	0.00019	3.90E-01	0.41	5
...CACGTT..	0.00030	7	1.2	0.00024	5.00E-01	0.3	5
tgccaa	0.00048	12	1.9	7.40E-07	1.50E-03	2.81	4

Genes	*PHO5, PHO8, PHO11, PHO84, PHO81*
Known motifs	*Factors*
CACGTGGG	*Pho4p (high affinity)*
CACGTTTT	*Pho4p (medium affinity)*

Feature-map of discovered patterns - PHO family

- The feature-map provides a convenient representation of the discovered patterns
  - Each colour represents one pattern.
  - Box height reflects pattern significance.
  - Clusters of mutually overlapping words represent sites larger than 6 bp.
- Green bars were superimposed, to indicate the positions of experimentally proven sites, and compare predictions with experimental knowledge.
  - For PHO11, no site is documented, we can thus not check the predictions.
  - For the other genes, the proven sites are detected as clusters of overlapping words

Site with experimental evidence

Clipping of upstream coding sequences

- In the particular case of the the yeast Saccharomyces cerevisiae, the initial annotations were over-predictive, and contained many false ORFs.
- Clipping upstream ORFs sometimes results in a loss of information.
- In the case of the PHO family, half of the know sites would be clipped, and the pattern discovery program would not identify any significant motif anymore.
- This problem has recently been solved, with the new annotations based on comparative genomics.

Real Site located in false ORF predictions

Hexanucleotide analysis of the MET family

Sequence	exp freq	occ	exp occ	P-value	E-value	sig	matching sequences
..ACGTGa	0.00033	13	2.9	1.00E-05	2.20E-02	1.67	9
.CACGTG.	**0.00012**	**13**	**1.0**	**6.90E-11**	**1.40E-07**	**6.84**	**9**
tCACGTG.	0.00033	13	2.9	1.00E-05	2.20E-02	1.67	9
tCACGTGa	consensus						
....TGTGGc	0.00027	10	2.3	1.50E-04	3.20E-01	0.49	7
...CTGTGG.	**0.00022**	**11**	**1.9**	**4.30E-06**	**8.90E-03**	**2.05**	**8**
..aCTGTG..	0.00036	12	3.1	9.90E-05	2.10E-01	0.69	9
.aaCTGT...	0.00063	17	5.4	4.90E-05	1.00E-01	0.99	11
aaaCTG....	0.00074	17	6.4	0.00037	7.60E-01	0.12	11
aaaCTGTGGc	consensus						
gcttcc	0.00039	12	3.4	0.00021	4.50E-01	0.35	7

Genes	*SAM2, MET6, MUP3, MET30, MET3, MET14, MET1, SAM1, MET17, ZWF1, MET2*
Known motifs	*Factors*
TCACGTG	*Cbf1p/Met4p/Met28p*
AAAACTGTGG	*Met31p; Met32p*

Feature-map of discovered patterns - MET family

- Two distinct motifs (combinations of words) are apparent.
  - blue-green TCACGTGA Met4p/Met28p/Cbf1p
  - red-violet AAACTGTG Met31p; Met32p
- Multiple clustered motifs ar sometimes found, but not always.

Expected frequency calibration

- The results of string-based pattern discovery depend drastically on the choice of a background model.
- Taking the MET family as example
  - With 6nt calibration in intergenic sequences, the Met4p binding site appears at rank 1, and Met31p at rank 3
  - With equiprobable nucleotides, Met4p only appears are rank 20, and Met31p at rank 32. In other terms, they will never be considered as the most interesting motifs
  - With a single-nucleotide calibration, the Met4p appears at rank 4 and Met31p at rank 13. The first motif would thus have been easily detected, but not the second one.

		Background model		
pattern	rev compl	intergenic	Bernoulli	equiprobable
atcacg....	....cgtgat	9	44	139
gtcacg....	....cgtgac	5	34	266
.tcacgt...	...acgtga.	2	4	20
..cacgtg..	..cacgtg..	1	3	23
...acgtga.	.tcacgt...	2	4	20
....cgtgac	gtcacg....	5	34	266
....cgtgat	atcacg....	9	44	139
gccaca....	....tgtggc	7	17	164
.ccacag...	...ctgtgg.	3	13	99
..cacagt..	..actgtg..	6	21	75
...acagtt.	.aactgt...	4	19	32
....cagttt	aaactg....	10	18	33
gcttcc	ggaagc	8	10	77

Effect of oligonucleotide size on the significance

		oligoncleotide length					
Family	**Pattern**	**4**	**5**	**6**	**7**	**8**	**9**
NIT	**aGATAAGa**	1.8	4.1	**9.1**	4.6	0.9	-
MET	**gTCACGTG**	4.4	4.1	7	**8.2**	3.2	-
	AAACTGTGg	1.5	2.3	1.6	4.8	**5.2**	4.9
PHO	**CACGTggg**	4.7	**8.4**	4.4	4.3	4.3	-
	aTGCCAA	**2.6**	1.5	**2.6**	0.6	-	-
	CTGCAC	-	-	1.7	-	-	-
INO	**CAACAAg**	2.9	2.1	**3.7**	1.3	-	-
	cCATGTGAA	-	-	2.7	3.2	**6.4**	0.4
PDR	**tCCGTGGa**	1.5	3.3	**7.4**	6.9	4.2	1.4
	tCCGCGga	6.9	**7.1**	4.5	5.6	1.8	1
GCN4	**GCNgtGACTCa**	5.4	**8.8**	8.2	7.7	4.7	-
	CAGCGGa	3.3	3.5	**4**	0.6	-	-
YAP	**CATTACTAA**	-	-	1	2.3	2.1	**3.2**
	cCGTTCC	0.1	0.5	**3.3**	0.3	-	-
YAP (400bp)	**cATTACTAA**	-	-	0.7	**4.5**	2.5	3.5
	cCGTTCC	0.8	0.5	**2.4**	0.7	0.2	-
TUP	**gtGGGGta**	**10.1**	9	8.6	5.6	3	-
	catAGGCAC	3.3	3.3	**4.3**	2.6	3.3	1.7

oligo-analysis results with known regulons (sig > 1)

Family	Factor	DNA-binding Domain	Known motifs	oligont	reverse oligont	score
NIT	GATA factors	Zn finger	GATAAG	TCTTATCT	AGATAAGA	20.0
MET	Cbf1p/Met4p/Met28p Met31p, Met32p	bHLH/bLZ/bLZ Zn finger	TCACGTG AAAACTGTGG	CACGTGAT CACGTGAC AACTGTGGCG	ATCACGTG GTCACGTG CGCCACAGTT	9.0 9.0 3.6
PHO	Pho4p (high affinity) Pho4p (medium affin.)	bHLH bHLH	GCACGTGGG GCACGTTTT	CCCACGTGCG AAACGTGCG TGCCAA CTGCAC	CGCACGTGGG CGCACGTTT TTGGCA GTGCAG	4.4 4.4 2.6 1.8
PDR	Pdr1p, Pdr3p	Zn_2Cys_6 binuclear cluster	tytCCGYGGary	TCCGTGGAA TCCGCGG	TTCCACGGA CCGCGGA	7.4 4.5
GCN4	Gcn4p	bZip	RRTGACTCTTT	ATGACTCA AGTGACTCA ATGACTCT ATGACTCC ATGACTA CCGCTG GCCGGT	TGAGTCAT TGAGTCACT AGAGTCAT GGAGTCAT TAGTCAT CAGCGG ACCGGC	8.5 8.5 8.5 8.5 3.8 3.7 1.3
INO	Ino2p/Opi1p	bHLH/leucine zipper	CATGTGAAWT	CAACAACG CAACAAG TTCACATG	CGTTGTTG CTTGTTG CATGTGAA	3.8 3.8 2.8
HAP 2/3/4	Hap2/3/4/5p		CCAAY	AGAGAGA	TCTCTCT	2.8
GAL4	Gal4p	Zn_2Cys_6 binucl. cluster	$CGGn_{11}CCG$	no significant pattern		

van Helden et al. (1998). *J Mol Biol* **281**(5), 827-42.

Hexanucleotide analysis of the GAL family

Sequence	exp freq	occ	exp occ	P-value	E-value	sig	matching sequences
agacat	0.00044	9	2.1	0.00033	**0.69**	**0.16**	4

Genes	*GAL1, GAL2, GAL7, GAL80, MEL1, GCY1*
Known motifs	*Factors*
CGGn$_5$wn$_5$CCG	*Gal4p*

- With the GAL family, the program returns a single pattern.
  - The significance of this pattern is very low.
  - This level of significance is expected at random ~ once per sequence set.
  - This can be considered as a negative result: the program did not detect any really significant pattern.
- Why did the program fail to discover the GAL4 motif ?

DNA/protein interface of the yeast transcription factor Gal4p

spaced pairs of trinucleotides
in upsteam sequences of the GAL family
observed occurrences
expected occurrences
CGGn11CCG
0
10
20
30
40
50
60

Dyad analysis of the GAL family

Sequence	exp freq	obs occ	exp occ	P-value	E-value	sig
..GGa.....CCG.	0.00006	10	0.5	2.70E-10	1.20E-05	4.92
.CGG.....Cga	0.00006	10	0.5	4.80E-10	2.10E-05	4.68
.CGG.....CCG.	**0.00007**	**20**	**0.6**	**2.10E-12**	**9.20E-08**	**7.03**
.CGG.....tCC..	0.00006	10	0.5	2.70E-10	1.20E-05	4.92
.CGG.....cgC...	0.00004	6	0.4	5.30E-06	2.30E-01	0.64
tCG.....CCG.	0.00006	10	0.5	4.80E-10	2.10E-05	4.68
cCG.....CCG.	0.00005	6	0.4	6.40E-06	2.80E-01	0.55
yCGGa.....ckCCGa						
AGA.....CCG	0.00010	8	0.9	7.00E-06	3.10E-01	0.51
CCG.GCG	0.00005	6	0.5	9.30E-06	4.00E-01	0.39

Genes	*GAL1, GAL2, GAL7, GAL80, MEL1, GCY1*
Known motifs	*Factors*
CGGn$_5$wn$_5$CCG	*Gal4p*

Feature-map of discovered patterns - GAL family

- Clusters of overlapping dyads indicates that conservation extends over 3 bp on each side of the dyad.
- Some genes, but not all, contain multiple motifs (synergic effect).

Dyad analysis: regulons of Zn cluster proteins

FACTOR	# genes	KNOWN MOTIFS	DYADS	REVERSE DYADS	SCORE
GAL4	6	CGGn$_{11}$CCG	TCGGAn$_9$TCCGG TCGGCGCAGAn$_4$TCCGG	CCGGAn$_9$TCCGA CCGGAn$_4$TCTGCGCCGA	7.8 7.8
HAP1	9	CGGnnntanCGG	GGAn$_5$CGGC GGGGGn$_{12}$GGC CCTn$_{10}$GGC	GCCGn$_5$TCC GCCn$_{12}$CCCCC GCCn$_{10}$AGG	1.8 1.4 1.1
LEU3	5	RCCggnnccGGY	CCGn$_3$CCG	CGGn$_3$CGG	1.0
LYS	6	wwwTCCrnyGGAwww	AAATTCCG TCCGCTGGA	CGGAATTT TCCAGCGGA	1.9 1.0
PDR	6	tytCCGYGGary	CTCCGTGGAA CTCCGCGGAA	TTCCACGGAG TTCCGCGGAG	6.7 6.7
PPR1	3	wyCGGnnwwykCCGaw		CGGn$_6$CCG	0.5
PUT3	2	yCGGnangcgnannnCCGa	CGGn$_{10}$CCG	CGGn$_{10}$CCG	1.2
UGA3	3	aaarccgcsggcggsawt	CGGn$_{14}$AGG GCCn$_{11}$TCC	CCTn$_{14}$CCG GGAn$_{11}$GGC	1.7 1.0
UME6	25	tagccgccga	TCGGCGGCTA	TAGCCGCCGA	4.9
CAT8	5	CGGnnnnnnGGA	CGGn$_4$ATGGAA	TTCCATn$_4$CCG	6.0

van Helden et al. (2000). *Nucleic Acids Res* **28**(8), 1808-18.

Comparison of discovered patterns with known sites (LYS family)

Patterns discovered by dyad analysis

Experimental measurement of activity

Regulatory Sequence Analysis

Quantitative evaluation of pattern discovery results

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Validation of pattern discovery with yeast regulons

- These figures regroup patterns detected with
  - *oligo-analysis*
  - *dyad-analysis*
- Regulons were collected from TRANSFAC and aMAZE.
- All the regulons with ≥5 genes were analyzed.
  - Significant patterns (sig ≥ 2) are detected in 65% of the regulons.
- As a negative control, sets of random genes were analyzed.
  - The rate of false positive follows pretty well the statistical expectation.

Simonis et al. (2004). Bioinformatics 20: 2370-2379

Pattern significance in regulons - Saccharomyces cerevisiae

- As a control, we compare the significance of patterns discovered
  - **in regulons** (*positive control*)
  - **in random gene selections** (*negative control*)
- In the yeast *Saccharomyces cerevisiae*
  - The rate of false positive corresponds remarkably well with the theoretical expectation.
  - When the score increases,
    - the sensitivity (patterns discovered in regulons) decreases,
    - the specificity increases (less patterns in random selections)

Sand, O., Turatsinze, J. V. and van Helden, J (2008). Evaluating the prediction of cis-acting regulatory elements in genome sequences In *Modern genome annotation: the BioSapiens network* (Springer).

Rate of false positive in different organisms

- The analysis of random gene selections allows to evaluate the rate of false positive returned by a pattern discovery program.
- The rate of false positive is good for microbes (bacteria, yeasts, ...), but increases for multicellular organisms (e.g. the fly Drosophila, the plant Arabidopsis thaliana, …).
- The rate of false positive is also higher in the protozoan Plasmodium falciparum (the agent of the malaria) than in bacteria and yeast.

Rate of false positive in higher organisms

- The rate of false positive increases dramatically with higher organisms.
- This is likely to come from
  - a bad treatment of repetitive elements : genome-scale calibration does not account for local frequencies
  - positional heterogeneities : oligonucleotide frequencies depend on the distance from the gene
  - the higher heterogeneity of genomic sequences in these organisms (GC-rich vs AT-rich promoters)
- We are currently developing more elaborate background models to treat this problem.

Pattern significance in regulons - Homo sapiens

- In Homo sapiens
  - The rate of false positive is much higher than the theoretical expectation
  - The number of patterns detected in regulons is still higher, but the significance score is quite inefficient to distinguish between reliable motifs and false positives.
  - This indicates that the background model is unadequate to treat the complexity of human promoters.

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String-based pattern discovery: strengths

- Deterministic (not heuristic) and exhaustive
  - all possible words/dyads are tested
  - ability to return several patterns in a single run
- Fast (2-3 seconds/family)
- Time increases linearly with sequence set
  - Can be applied to very large sequence sets (full genomes)
- Ability to return a negative answer
  - "not a single over-represented pattern in this sequence set"
  - Corollary: very low false positive rate
- Pattern assembly refines the result
  - ability to detect some level of degeneracy
    (result contains words differing by single substitutions)
  - ability to detect motifs larger than the oligonucleotide size
    (result contains strongly overlapping words)

String-based pattern discovery: weaknesses

- No direct treatment of pattern degeneracy
  - NB: degenerated words can be analyzed with similar statistics, but it is not tractable due to the increase of the number of patterns: 15k possible words of length k.
- String patterns are poor descriptions for genome-scale pattern matching.
  - Matrices are more appropriate to describe the weight of each substitution at a given position.
- Solution
  - string-approach for pattern discovery
  - use discovered strings as seeds for building a matrix, which can be used for pattern search

Examples of applications

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Analysis of cell cycle data : results

family	oligo-analysis				dyad-analysis (non-coding dyad frequency calibration)			
	word	reverse clpt	sig	remark	dyad	reverse clpt	sig	remark
CLN2	TACGCGAA.	.TTCGCGTA	30.5	MBF; SBF variant	TTTACGCGAAAA	TTTTCGCGTAAA	29.0	MBF; SBF variant
	TACGCGTA.	.TACGCGTA	30.5	MBF; SBF	GAAAACGCGTAAA	TTTACGCGTTTTC	29.0	MBF; SBF
	TTCGCGTCG	CGACGCGAA	30.5	MBF; SBF variant	TTTTCGCGTCA.	.TGACGCGAAAA	29.0	MBF; SBF variant
	AAACGCGAA.	.TTCGCGTTT	30.5	MBF; SBF variant	TTTACGCGTCA.	.TGACGCGTAAA	29.0	MBF; SBF
	TTCGCGTCA.	TGACGCGAA	30.5	MBF; SBF variant	CGACGCGAAAA	TTTTCGCGTCG	29.0	MBF; SBF variant
	TGCCAA	TTGGCA	1.8		GAAAACGCGTCA.	.TGACGCGTTTTC	8.1	MBF; SBF
	ATCAAG	CTTGAT	1.3		AAAn8CGC	GCGn8TTT	1.9	
					CAAn5CGC	GCGn5TTG	1.1	
Y' (purged)	CTCGTC	GACGAG	1.8		AGTnGAG	CTCnACT	3.0	
	AGTATC	GATACT	1.2		CAGn{10}ATC	GATn{10}CTG	2.0	
					ATCn{12}GAG	CTCn{12}GAT	1.2	
histone (purged)	CGCCCG	CGGGCG	2.6		GCGn8AGAAC	GTTCTn8CGC	3.0	
	CCAGAA	TTCTGG	1.7	Mcm1	CGCCCG	CGGGCG	1.3	
					ATTn2GCG	CGCn2AAT	1.3	
Cell cycle MET	TGCCACAGTT	AACTGTGGCA	10.1	Met31; Met32	GCCACAGTT	AACTGTGGC	8.6	Met31; Met32
	TCACGTGA	TCACGTGA	10.1	Met4/Met28/Cbf1	GTCACGTGAC	GTCACGTGAC	6.9	Met4/Met28/Cbf1
	ACAGAG	CTCTGT	1.9					
	GACTCA	TGAGTC	0.9					
CLB2	CCAAAG	CTTTGG	1.3		CCCn6GAA	TTCn6GGG	2.5	ECB
	CCTTCA	TGAAGG	0.9	NEG	CAAn13GCC	GGCn13TTG	0.9	
					ACCn14AAT	ATTn14GGT	0.9	
MCM	AGAGCA	TGCTCT	1.4		TCCCn4GGGA	TCCCn4GGGA	3.9	ECB variant
	TCCTAA	TTAGGA	1.0	Mcm1	AAAnAGG	CCTnTTT	2.8	ECB ?
					AGGn10ACT	AGTn10CCT	1.2	
SIC1	AACCAGCAA	TTGCTGGTT	20.0	Swi5;Ace2	AACCAGCA.	. . . .TGCTGGTT	20.0	Swi5; Ace2
	AGCCAGCAA	TTGCTGGCT	20.0	Swi5;Ace2	AACCAGCCAGCA	TGCTGGCTGGTT	20.0	Swi5; Ace2
	AACCAGCC	GGCTGGTT	8.0	Swi5;Ace2				

Gene clusters from Spellman et al. (1998). Mol Biol Cell 9(12), 3273-97
Pattern discovery : van Helden et al. (2000). Nucleic Acids Res 28: 1808-1818.

Plasmodium erythrocytic cycle

figure 2: Cycle de vie de Plasmodium falciparum (source :institut pasteur (France) page web)

Bozdech et al. (2003). The Transcriptome of the Intraerythrocytic Developmental Cycle of Plasmodium falciparum. PLoS Biol 1: E5.

Over-represented oligos in promoters of under- and over-expressed genes at different time points of the erythrocytic cycle of Plasmodium falciparum

Essaghir, Vaquero & van Helden, in prep

Erythrocytic cycle in Plasmodium falciparum
Under- and over-expressed oligonucleotides in random gene selections

Essaghir, Vaquero & van Helden, in prep

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O.Sand & J.van Helden, in prep.

Regulatory Sequence Analysis

Supplementary material

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Pattern discovery: string-based algorithms

- Count occurrences observed for each word
- Calculate expected word frequencies
  - Choice of a model :
    - independently distributed nucleotides (equiprobable or biased alphabet utilization)
    - Markov chain : on basis of subword frequencies
    - External reference (e.g. word frequencies observed in the whole set of upstream sequences)
- Calculate a score for each word
    - obs/exp ratio (very bad)
    - log-likelihood
    - Z-value
    - binomial probability
- Select all words above a defined threshold
  - Statistical criterion for establishing the threshold