Pattern Discovery in Biosequences

ISMB 2002 tutorial

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Latest version of the slides at http://www.cs.ucr.edu/~stelo/ismb02/

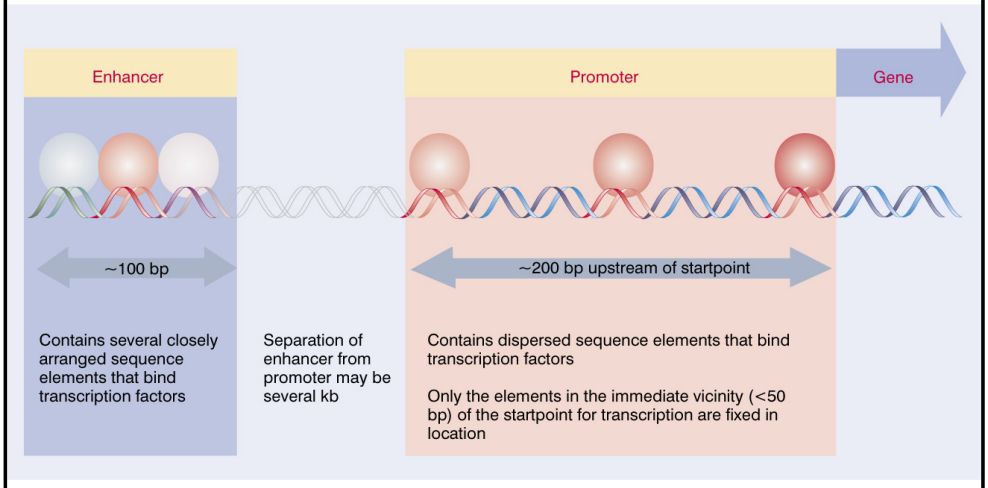
Roadmap

- Why “pattern discovery”
- Basic concepts
- Problem definition
- Classification of patterns
- Complexity results
- Efficient algorithms for pattern discovery
  - Deterministic patterns: Verbumculus
  - Rigid patterns: Teiresias, Winnower, Projection, Weeder
  - Profiles: Gibbs sampling, Meme
- Appendix

Why “pattern discovery”?

Discovery of regulatory elements

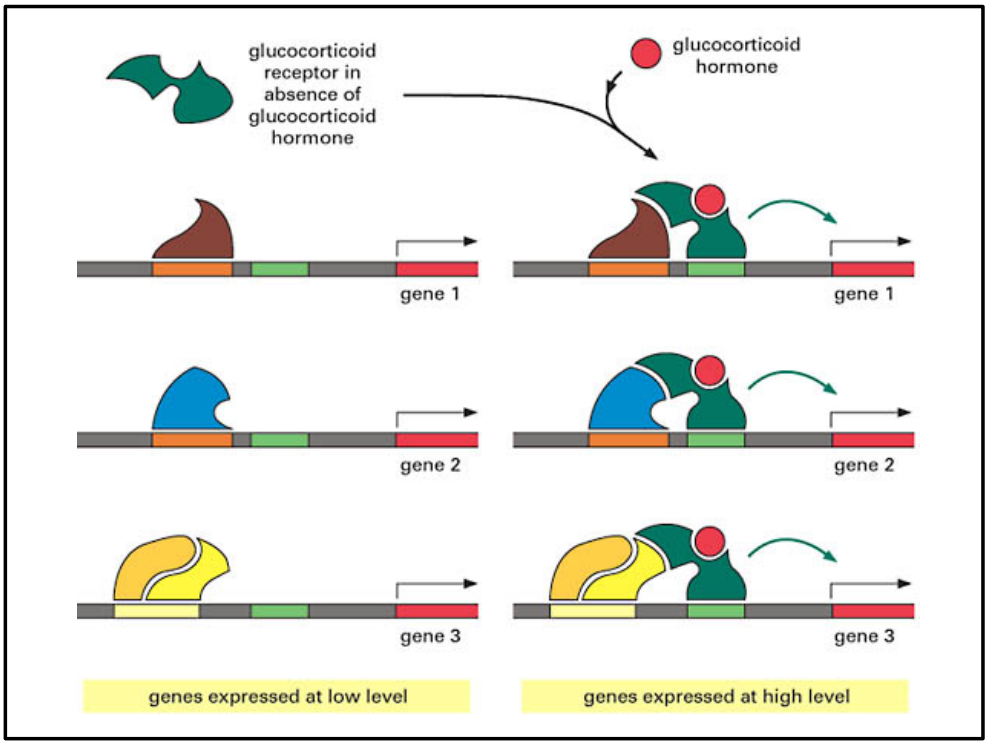
- *Promoter:* a region of DNA involved in binding of RNA polymerase to initiate transcription
- *Enhancer:* a region of DNA that increases the utilization of (some) promoters (it can function in either orientation and any location relative to the promoter)
- *Repressor:* a region of DNA that decreases the utilization of (some) promoters

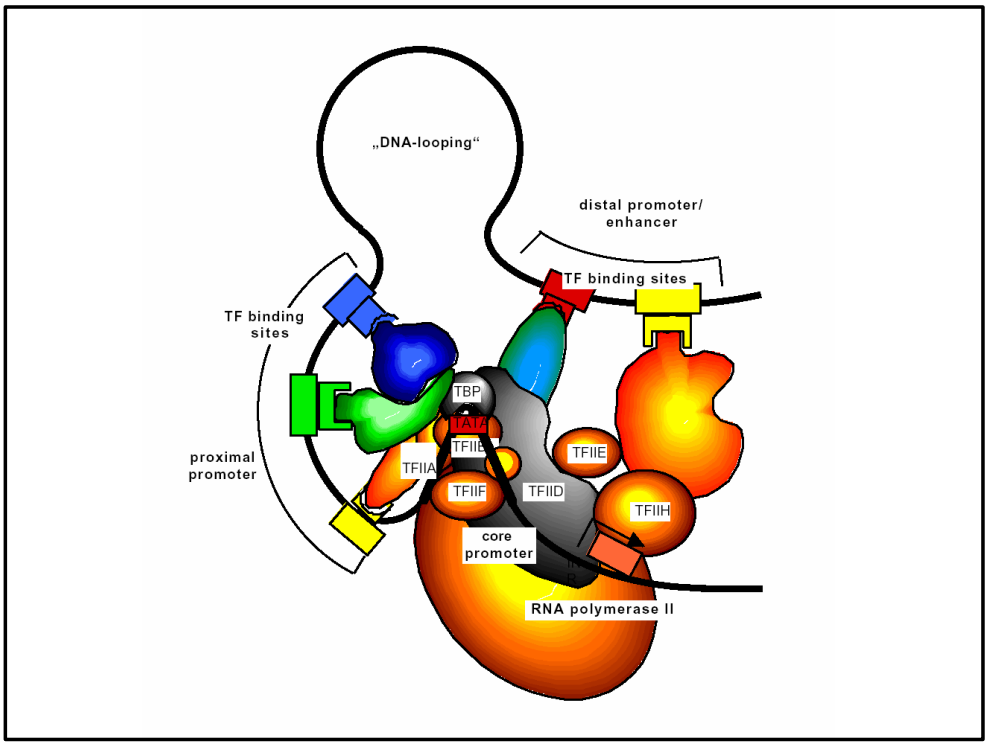


Source: Lewin, genes VII

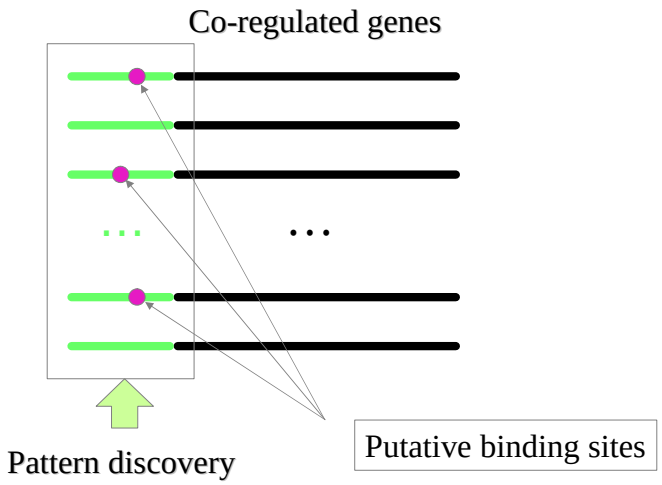
Transcription

- Different factors are involved in the transcription machinery
  - presence of transcription factors and their binding sites
  - ability of DNA to bend
  - relative location of the binding sites
  - presence of CpG islands (“p” is for phosphate)
  - …


glucocorticoid receptor in absence of glucocorticoid hormone
glucocorticoid hormone
gene 1
gene 1
gene 2
gene 2
gene 3
gene 3
genes expressed at low level
genes expressed at high level


„DNA-looping“
distal promoter/ enhancer
TF binding sites
TF binding sites
TBP
TATA
TFIIB
TFIIA
TFIIE
TFIIF
TFIID
TFIIH
proximal promoter
core promoter
RNA polymerase II

Transcription factors binding sites



Basic concepts

Some notations

Σ : alphabet

$a, b, c, \ldots$: symbols from Σ

x : sequence/string over Σ, $|x| = n$

$\{x_1, x_2, \ldots, x_k\}$: multi-sequence, $\sum_{i=1}^{k} |x_i| = n$

y (or w) : substring of x, $|y| = m$

y^i : the substring $\underbrace{yy \cdots y}_{i \text{ times}}$ $(i \geq 0)$

Some notations

$y_{[i]}$: the i-th symbol of y $(1 \leq i \leq m)$

$y_{[i,j]}$: the string $y_{[i]} y_{[i+1]} \cdots y_{[j]} (1 \leq i \leq j \leq m)$

$y_{[1,j]}$: are the prefixes of y $(1 \leq j \leq m)$

$y_{[j,m]}$: are the suffixes of y $(1 \leq j \leq m)$

Occurrences vs. Colors

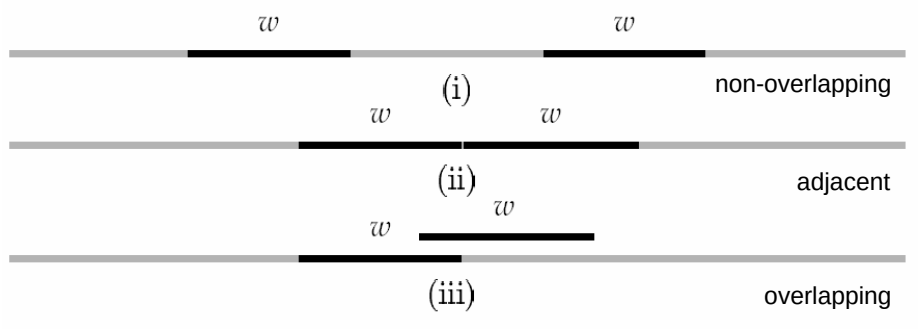
Some notations

$f(y)$: number of occurrences of y

$c(y)$: number of colors of y

Depending on the application, $f(y)$ and/or $c(y)$ is used. In general, these quantities are called the support of y.

Occurrences: types



For our purposes, any of the above is simply an occurrence
Keep in mind that in some cases you may have to distinguish them

Example (DNA)

x_1 = **CCACCCTTTTGTGGGGCTTCTATTTCAAGG**

x_2 = **TTGTTCTTCCTGCATGTTGCGCGCAGTGCG**

x_3 = **TTCTAAAAGGGGCATTATCAGAAAAAGAAG**

x_4 = **GTGTAAAATTGTGTGCTACCTACCGTATTA**

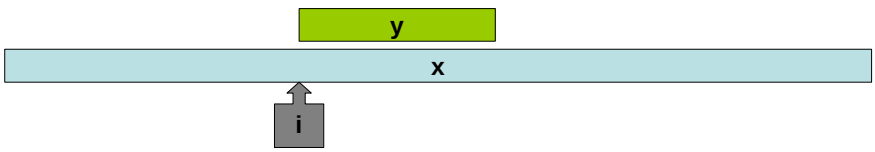
- Σ = {**A,C,G,T**} $|\Sigma| = 4$ $n = 120$
- e.g., y = **AAAA** is a substring of x_3 and x_4
  - $f(y) = 4$ (occurrences can overlap)
  - $c(y) = 2$

Bernoulli and Markov models

- Two typical hypothesis about the source (probabilistic models)
- *Bernoulli*: symbols are generated independently and they are identically distributed (*i.i.d.* or *memoryless*)
- *Markov*: the probability distribution for the "next" symbol depends on the previous *h* symbols (*h>0* is the *order* of Markov chain)

Example (no. of occurrences)

- We want to describe the number of occurrences *f(y)* of a pattern *y* in the text *x*
- Recall $|x|=n$, $|y|=m$
- Let us choose a particular location *i* in the text *(i < n-m+1)*



Example on no. of occurrences

- Assuming a Bernoulli model for the source that generated *x*, i.e., symbols are generated i.i.d., the probability that *y* occurs at position *i* in the text is
  $P(x_{[i]}=y_{[1]}, x_{[i+1]}=y_{[2]},\ldots,x_{[i+m-1]}=y_{[m]})=$
  $P(x_{[i]}=y_{[1]})\, P(x_{[i+1]}=y_{[2]}) \ldots P(x_{[i+m-1]}=y_{[m]})=$
  $p_{y[1]}\, p_{y[2]} \cdots p_{y[m]}$
- Note that in general we do not know the "true" p_a and they have to be estimated from the observation *x*

Example on no. of occurrences

- Now we have to consider that *y* can occur in $n-m+1$ positions (from 1 to $n-m+1$)
- At each position the probability is $p_{y[1]}\, p_{y[2]} \ldots p_{y[m]}$
- Since we assumed a Bernoulli model, the random variables for each position are independent, then

$$E[X] = (n-m+1) \prod_{j=1..m} p_{y[j]}$$

A r.v. for the no. of occurrences

Let Z_y be a r.v. for the number of occurrences of y, p_a be the probability of $a \in \Sigma$, and $|y| = m \leq (n+1)/2$, then

- $E(Z_y) = (n-m+1)\prod_{i=1}^{m} p_{y_{[i]}} = (n-m+1)\,\hat{p}$
- $Var(Z_y) = E(Z_y)(1-\hat{p}) - \hat{p}^2(n-m+1)(n-m) + 2\hat{p}B(y)$

where $B(y) = \sum_{d \in P(y)} (n-m+1-d) \prod_{i=m-d+1}^{m} p_{y_{[i]}}$

and $P(y)$ is the set of period lengths of y

A r.v. for the no. of colors

Let W_y be a r.v. for the number of colors of y in $\{x_1, x_2, \ldots, x_k\}$, and Z_y^i be the random variable of occurrences of y in the i-th sequence $(1 \leq i \leq k)$, then

- $E(W_y) = k - \sum_{i=1}^{k} P[Z_y^i = 0]$

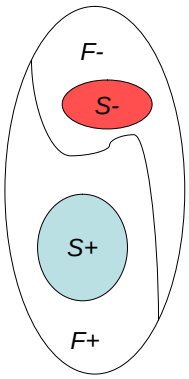
(because $E(W_y) = P[Z_y^i > 0]$)

“Pattern Discovery”: the problem

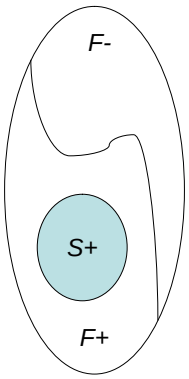
Pattern discovery: the problem

- Given a set of sequences S^+ and a model of the source for S^+
- Find a set of patterns in S^+ which have a support that is statistically significant with respect to the probabilistic model
- If we are also given negative examples S^-, we must ensure that the patterns do not appear in S^-

Pattern discovery problem

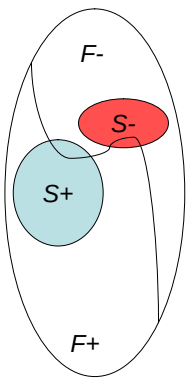


Pos & Neg examples

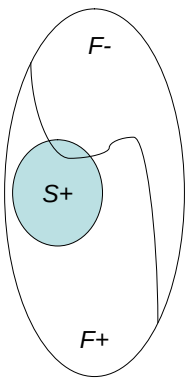


Only Pos examples

Noisy data



Pos & Neg examples



Only Pos examples

Pattern discovery “dimensions”

- Type of learning
  - from positive examples only (unsupervised)
  - from both positive and negative examples (supervised)
  - noisy data
- Type of patterns
  - deterministic, rigid, profiles, …
- Measure of statistical significance
- *A priori* knowledge

Output

- *True positive*: a pattern belonging to the positive training set which has been correctly classified
- *True negative*: a pattern belonging to the negative training set which has been correctly classified
- *False positive:* misclassified as positive
- *False negative*: misclassified as negative

Measuring pattern discovery

- *Complete:* if no true pattern is missed
  - but we may report too many patterns
- *Sound:* if no false pattern is reported
  - but we may miss true positives
- Usually there is a tradeoff between soundness and completeness: if you increase one, you will decrease the other

Measuring the performance

- Information Retrieval measures
  - *Precision = true pos / (true pos + false pos)*
    - expresses the proportion of discovered patterns out of the total reported positive
    - also called *sensitivity*
  - *Recall = true pos / (true pos + true neg)*
    - expresses the proportion of discovered patterns out of the total of true patterns

A classification of patterns

Types of patterns

- Deterministic patterns
- Rigid patterns
  - Hamming distance
- Flexible patterns
  - Edit distance
- Matrix profiles

✓ A *motif* is any of these patterns, as long as it is associated with biological relevance

Deterministic Patterns

- Definition: *Deterministic patterns* are strings over the alphabet Σ
  - e.g., "**TATAAA**" (TATA-box consensus)
- Discovery algorithms are faster on these types of patterns
- Usually not flexible enough for the needs of molecular biology

Rigid patterns

- Definition: Rigid patterns are patterns which allow substitutions/"don't care" symbols
  - e.g., the patterns under IUPAC alphabet {**A,C,G,T,U,M,R,W,S,Y,K,V,H,D,B,X,N**} where for example **R**=[**A**|**G**], **Y**=[**C**|**T**], etc.
  - e.g, "**ARNNTTYGA**" under IUPAC means "**A**[**A**|**G**][**A**|**C**|**G**|**T**][**A**|**C**|**G**|**T**]**TT**[**C**|**T**]**GA**"
- Note that the size of the pattern is not allowed to change

Hamming distance

- Definition: Given two strings *y* and *w* such that $|y|=|w|$, the Hamming distance *h(w,y)* is given by the number of mismatches between *y* and *w*
- Example:
  y=**GATTACA**
  w=**TATAATA**
  h(w,y)=h(y,w)=3

Hamming neighborhood

- Definition: Given a string *y*, all strings at Hamming distance at most *d* from *y* are in its *d-neighborhood*

- Fact: The size *N(m,d)* of the *d*-neighborhood of a string *y*, $|y|=m$, is

$$N(m,d)=\sum_{j=0}^{d}\binom{m}{j}\left(|\Sigma|-1\right)^{j}\in O\left(m^{d}\,|\Sigma|^{d}\right)$$

Hamming neighborhood

- Example:
  y = **ATA** the 1-neighborhood is
  {CTA,GTA,TTA,
  AAA,ACA,AGA,
  ATC,ATG,ATT,
  ATA}

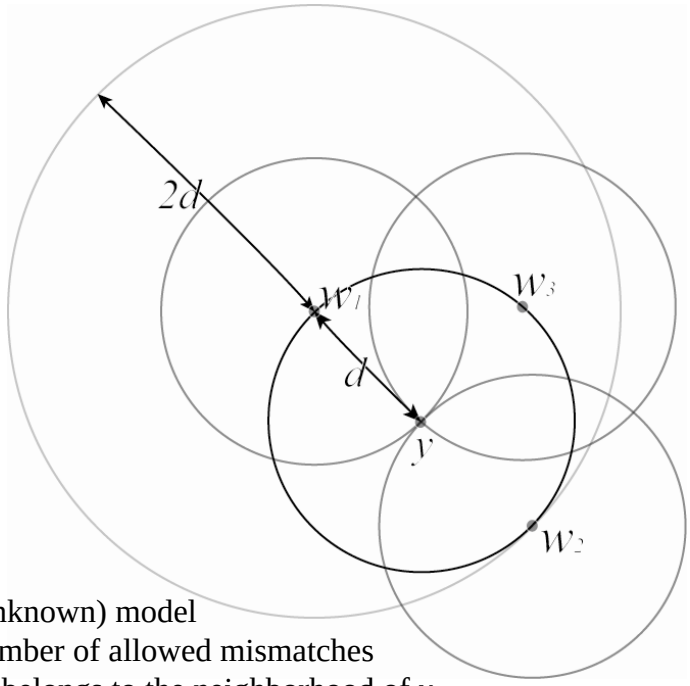
- This set can be written as a rigid pattern
  {NTA|ANA|ATN}

Models

- We may be able to observe occurrences of the neighbors of *y*, but we may never observe an occurrence of *y*

- Definition: The center of the *d*- neighborhood *y* is also called the *model*

- Definition: We say that a model is *valid* if it has enough support (occurrences/colors)



y is the (unknown) model
d is the number of allowed mismatches
w_1, w_2, w_3 belongs to the neighborhood of *y*

Hamming neighborhood

- Fact: Given two strings w_1 and w_2 in the *d*-neighborhood of the model *y*, then $h(w_1,w_2) \leqslant 2d$
- The problem of finding *y* given $w_1,w_2,\ldots$ is sometimes called *Steiner sequence* problem
- Unfortunately, even if we were able to determine exactly all the w_i in the neighborhood, there is no guarantee to find the unknown model *y*

Example

- Suppose $m=4$, $d=1$ and that we found occurrences of **{AAAA,TATA,CACA}**
- The pairwise Hamming distance is 2 but there is no string at Hamming distance 1 to each of these

Word match filtering

- Fact: Given two strings w_1 and w_2 in the d-neighborhood of the model y, they both contain an occurrence of a word of length at least $\lfloor m/(2d+1) \rfloor$
- Example: y = **GATTACA**
  w_1 = **GATTTCA**
  w_2 = **GGTTACA**
  TT and **CA** are occurring exactly. In fact $\lfloor m/(2d+1) \rfloor = \lfloor 7/3 \rfloor = 2$

Word match filtering

- Proof: there are at least *m-2d* matching positions, divided into at most *2d+1* segments (some possibly of length *0*) by intervening mismatches. The average length of a segment is therefore *(m-2d)/(2d+1)*.
  Hence there exists a segment with no mismatches of length at least $\lceil (m-2d)/(2d+1) \rceil = \lfloor m/(2d+1) \rfloor$.

Flexible patterns

- Definition: *Flexible patterns* are patterns which allow substitutions/"don't care" symbols and variable-length gaps
  - e.g., Prosite **`F-x(5)-G-x(2,4)-G-*-H`**
- Note that the length of these pattern is variable
- Very expressive
- Space of all patterns is huge

Edit distance

- Definition: the *edit distance* between two strings *y* and *w* is defined as the minimum number of edit operations - insertions, deletions and substitutions - necessary to transform *y* into *w* (matches do not count)
- Definition: a sequence of edit operations to transform *y* into *w* is called an *edit script*

Edit distance

- The *edit distance problem* is to compute the edit distance between *y* and *w*, along with an optimal edit script that describes the transformation
- An alternative representation of the edit script is the *alignment*

Example

- Given *w* = **GATTACA**

  y = **TATATA**

  GATTACA►ATTACA►TTACA

  ►TATACA►TATATA (1 ins, 2 del, 1 sub)

  GATTACA►TATTACA

  ►TATACA►TATATA (0 ins, 1 del, 2 sub)

- Edit distance is 3

Corresponding global alignment

- Given *w* = **GATTACA**

  y = **TATATA**

- We can produce the following *alignments*

  GAT-TAC-A **G-ATTAC-A**

  --TATA-TA **-TAT-A-TA**

  where "**–**" represents a *space* (we cannot have "**–**" aligned with "**–**")

Profiles

- *Position weight matrices*, or *profiles*, are $|\Sigma| \times m$ matrices containing real numbers in the interval *[0,1]*
  - e.g.

A	*0.26*	*0.22*	*0.00*	*1.00*	*0.11*
C	*0.17*	*0.18*	*0.59*	*0.00*	*0.35*
G	*0.09*	*0.15*	*0.00*	*0.00*	*0.00*
T	*0.48*	*0.45*	*0.41*	*0.00*	*0.54*

  - *consensus*

Profiles as classifiers

- Profiles can be used directly to implement very simple classifiers

- Suppose we have a sample *S+* of known sites, and a sample *S-* of non-sites
- Given a new sequence *x*, how do we classify *x* in *S+* or in *S-?*

Example: CRP binding sites

- S+={**TTGTGGC, ACGTGAT, CTGTGAC,**
  TTTTGAT, ATGTGAG, ATGAGAC,
  AAGTGTC, TTGTGAG, TTGTGAG}
  ATTTGCA, CTGTAAC,
  CTGTGCG, CTGTAAC,
  ATGCAAA, TTGTGAC,
  GTGTTAA, GCCTGAC,
  ATTTGAA, TTGTGAT,
  TTGTGAT, TTGTGAT,
  ATTTATT, GTGTGAA,

Cyclic AMP receptor protein TFs in E.coli [Stormo & Hartzell, 89]

Training (CRP sites)

- Assume a Bernoulli model for each position

A	0.350	0.043	0.000	0.043	0.130	0.830	0.260
C	0.170	0.087	0.043	0.043	0.000	0.043	0.300
T	0.130	0.000	0.780	0.000	0.830	0.043	0.170
G	0.350	0.870	0.170	0.910	0.043	0.087	0.260

- Assume the uniform Bernoulli model for the non-sites *S-*, that is $p_A=0.25$, $p_C=0.25$, $p_T=0.25$, $p_G=0.25$ for all the positions

Testing

- Suppose you get x = **GGTGTAC**
  Is x more likely to belong to $S+$ or to $S-$?
  In other words, it is more likely to be generated from the Bernoulli model for $S+$ or from the uniform Bernoulli model (for $S-$)?
- Let's compute the probability

$$P(x = GGTGTAC \mid S+) = .35*.87*.78*.91*.83*.83*.3 = 0.045$$

$$P(x = GGTGTAC \mid S-) = (.25)^7 = 0.0000061$$

$$LR(x) = P(x \mid S+) / P(x \mid S+)$$

Discovering Deterministic Patterns

Enumerative approach: idea

- Define the search space
- List exhaustively all the patterns in the search space
- Compute the statistical significance for all of them
- Report the patterns with the highest statistical significance

Enumerative approach

- E.g., search space for deterministic patterns of size m is $O(|\Sigma|^m)$
- Can we do better than $|\Sigma|^m$?

Enumerative approach

- The search space for deterministic pattern is already too big
  - e.g., there are *1,048,576* possible deterministic patterns of size 10 on the DNA alphabet
- How to prune it?

Detection of Unusual Patterns

Naïve approaches

1) Enumerate and test all patterns composed by m symbols, for $1 \leqslant m \leqslant n$

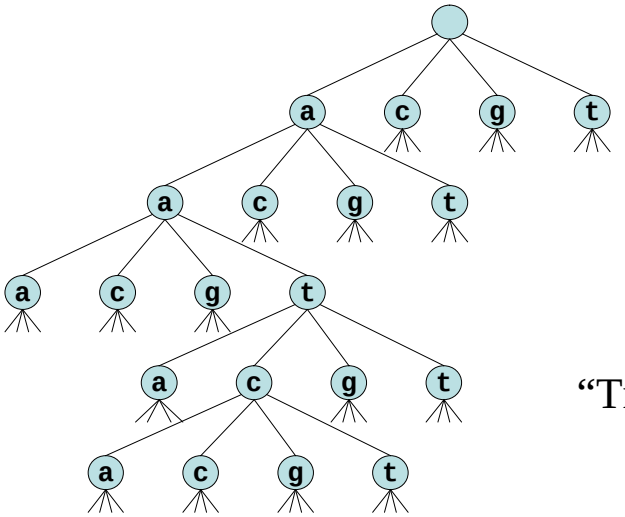
2) Enumerate and test all patterns which occur in the sequences

$n = 1{,}000{,}000 \quad |\Sigma| = 4$

1) Patterns to be tested $O(|\Sigma|^n)$
in this case $\propto 4^{1{,}000{,}000}$

2) Patterns to be tested $O(n^2)$
in this case $\propto 1{,}000{,}000^2$

Enumerating the $O(n^2)$ patterns



"Trie"

Basic operations on the trie

(*"trie"* comes from information re*trie*val)

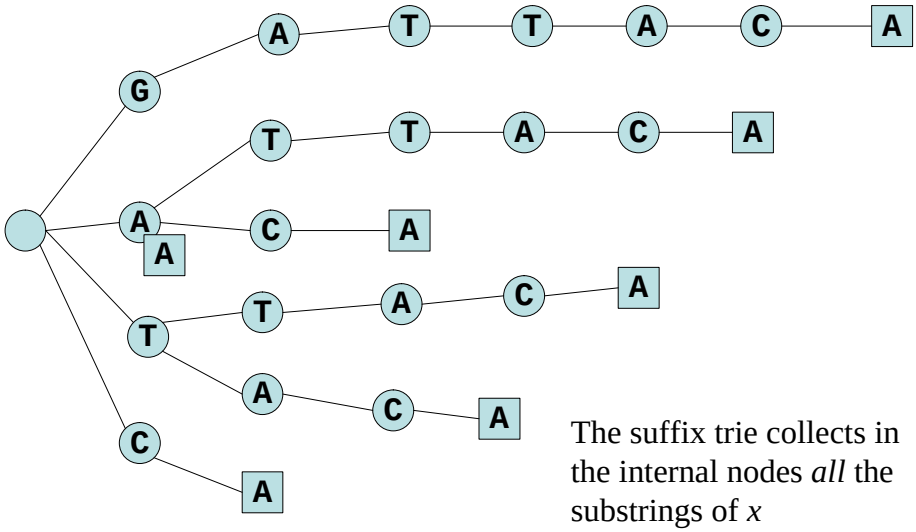
- *Construction*
- *Traversals*
  - Breadth-first
  - Depth-first
- *Query*
  - Given a pattern of length *m*, we can check if it belongs to the trie in time *O(m)*

Suffix trie

- We build a trie with all the suffixes of the text *x*
- Example: if *x* = **GATTACA** we use

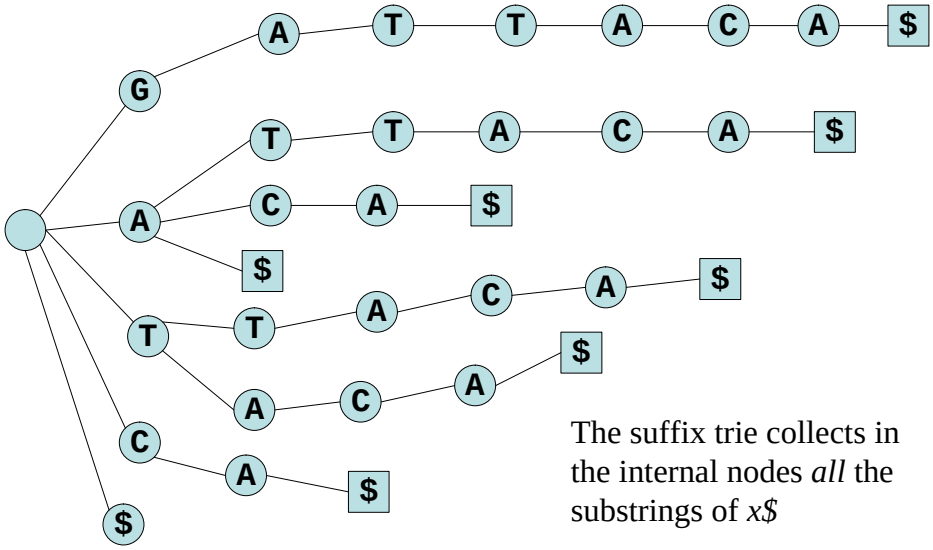
  GATTACA
  ATTACA
  TTACA
  TACA
  ACA
  CA
  A

Suffix trie for "**GATTACA**"



The suffix trie collects in the internal nodes *all* the substrings of *x*

Suffix trie for "**GATTACA$**"



The suffix trie collects in the internal nodes *all* the substrings of *x$*

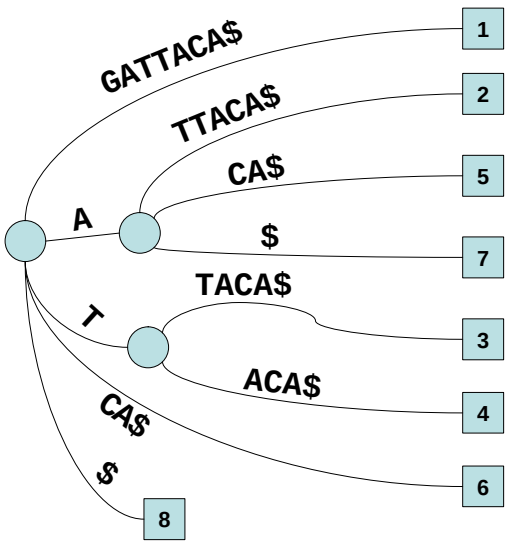
Suffix trie

- Construction $O(n^2)$
- Space $O(n^2)$
- Query $O(m)$

- We can do better by removing unary nodes from the tree, and coalescing the edges
- The result is called *suffix tree*

Suffix tree for "**GATTACA$**"

1 2 3 4 5 6 7 8



The suffix tree collects in the *implicit* internal nodes *all* the substrings of *x$*

The *locus* of a string is the node in the tree corresponding to it

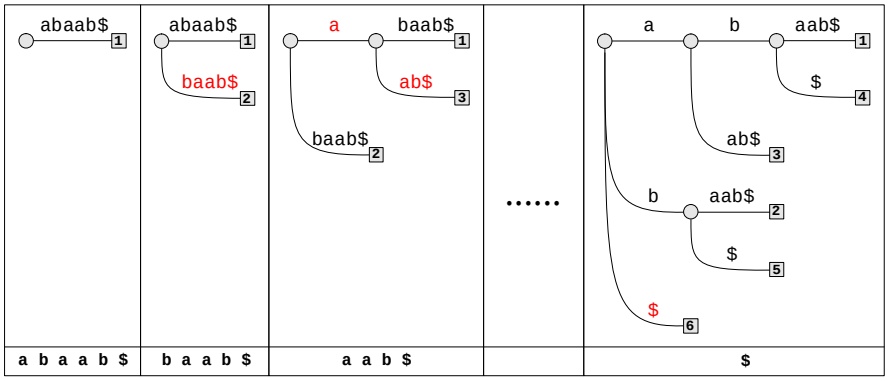
The label in the leaves identifies the suffix position (used to find pos all occs)

The number of leaves in the subtree corresponds to the number of occurrences

Space analysis

- Every node is branching
- The number of leaves is *n*
- Therefore the overall number of nodes is at most *2n-1*
- Use two integers (constant space) to identify labels on the arcs
- Therefore the overall size of the tree is *n*
- Note: we assume the standard RAM model that *log n* bits can be read, written or compared in constant time

Brute force construction



Worst case $O(n^2)$

1 2 3 4 5 6
a b a a b $

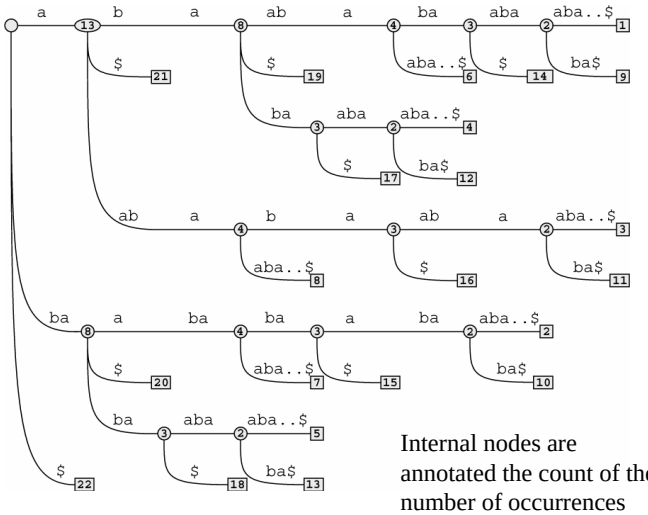
Average case $O(n \log n)$

Computing number of occurrences

ANNOTATE_$f(w)$ (**suffix_tree** T)
  for each leaf u of T **do**
    let $f(L(u)) = 1$
  visit T in depth-first traversal, for each internal node u **do**
    let $f(L(u))$ equal to the sum of $f(\cdot)$ of the children of u

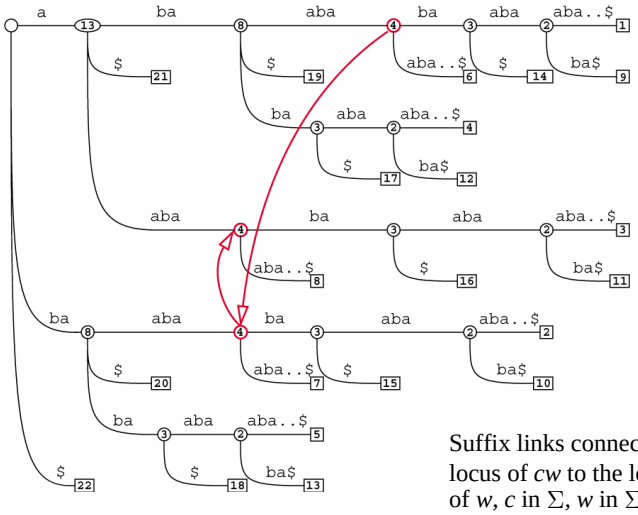
Time complexity is $O(n)$

Suffix tree for “**abaababaabaababaababa$**”



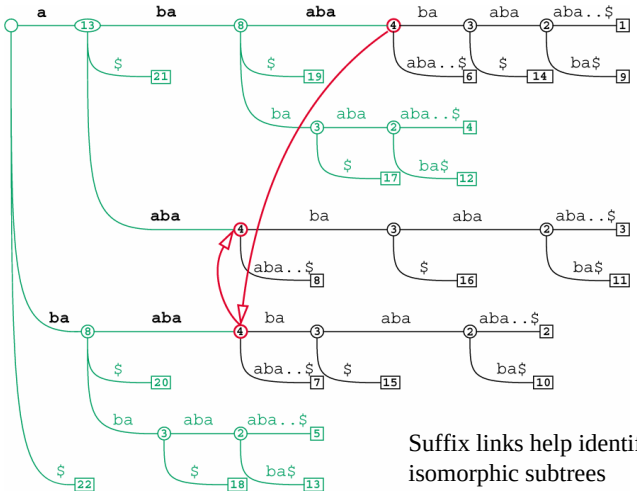
Internal nodes are annotated the count of the number of occurrences

Suffix links



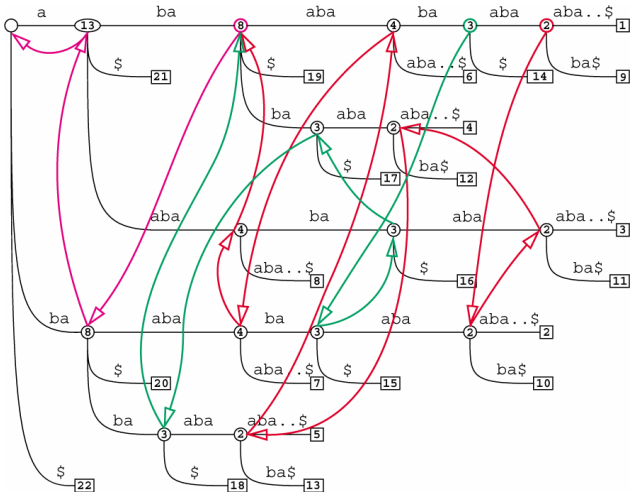
Suffix links connect the locus of *cw* to the locus of *w*, *c* in Σ, *w* in Σ*

Suffix links



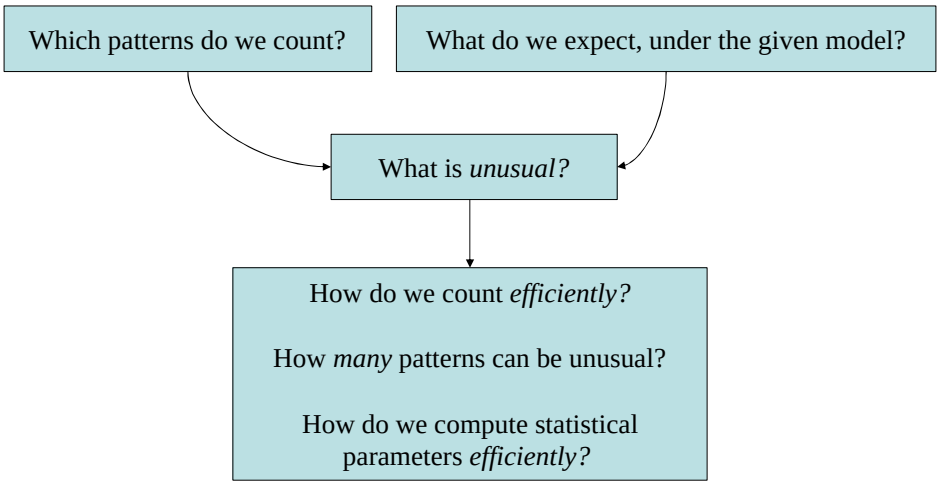
Suffix links help identifying isomorphic subtrees

All the suffix links (except leaves)



Suffix trees in a nutshell

- Suffix trees can be built in *O(n)* time and space [Weiner73, McCreight76, Ukkonen95, Farach97]
- Number of occurrences can be computed in *O(n)* time
- Number of colors can be computed in *O(n)* time [Hui92, Muthu02]



Scores based on occurrences

$$z_1(y) = f(y) - E(Z_y)$$

$$z_2(y) = \frac{f(y) - E(Z_y)}{\sqrt{E(Z_y)}}$$

$$z_3(y) = \frac{f(y) - E(Z_y)}{\sqrt{E(Z_y)\ (1-\hat{p})}}$$

$$z_4(y) = \frac{f(y) - E(Z_y)}{\sqrt{Var(Z_y)}}$$

where Z_y is a r.v. for the number of occurrences of y

Scores based on colors

$$z_7(y) = c(y) - E(W_y)$$

$$z_8(y) = \frac{c(y) - E(W_y)}{\sqrt{E(W_y)}}$$

where W_y is a r.v. for the number of colors of y

What is “unusual” ?

Definition:

Let y be a substring of x and $T \in \mathbb{R}^+$

- if $z(y) > T$, then y is over-represented
- if $z(y) < -T$, then y is under-represented
- if $|z(y)| > T$, then y is unusual

Problem

Given

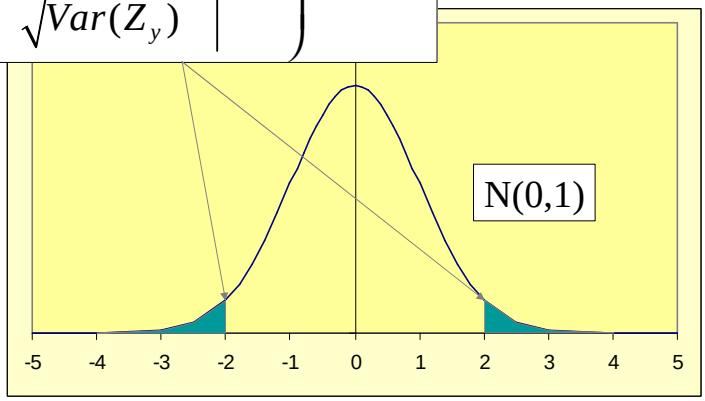
- Single/multi sequence x
- Type of count (f or c)
- Score function z
- Threshold T

Find

- The set of all unusual patterns in x w.r.t. *(f/c,z,T)*

How to choose the threshold

$$P\left(\left|\frac{f(y)-E(Z_y)}{\sqrt{Var(Z_y)}}\right|>2\right)=.0456$$



Computational Problems

- Counting “events” in strings
  - occurrences
  - colors
- Computing expectations, variances, and scores (under the given model)
- Detecting and visualizing unusual patterns

Combinatorial Problem

- A sequence of size n could have $O(n^2)$ unusual patterns
- How to *limit* the set of unusual patterns?

Theorem:

Let C be a set of patterns from text x. If $f(y)$ remains constant for all y in C, then any score of the type

$$z(y) = \frac{f(y) - E(y)}{N(y)}$$

is monotonically increasing with $|y|$ provided that

- $N(y)$ is monotonically decreasing with $|y|$
- $E(y)/N(y)$ is monotonically decreasing with $|y|$

Theorem:

Score functions

$$z(y) = f(y) - E(Z_y), \qquad z(y) = c(y) - E(W_y),$$

$$z(y) = \frac{f(y) - E(Z_y)}{\sqrt{E(Z_y)}}, \qquad z(y) = \frac{c(y) - E(W_y)}{\sqrt{E(W_y)}},$$

$$z(y) = \frac{f(y) - E(Z_y)}{\sqrt{E(Z_y)(1-\hat{p})}},$$

are monotonically increasing with $|y|$, for all y in class C

Theorem:

If $p_{\max} < \min\left\{1/\sqrt[|y|]{4|y|}, \sqrt{2}-1\right\}$, then

$$z(y) = \frac{f(y) - E(Z_y)}{\sqrt{Var(Z_y)}}$$

is monotonically increasing with $|y|$, for all y in class C

abaababaabaababaababa

abaababaabaababaababa
aa aa

abaababaabaababaababa

baa baa

abaababaabaababaababa

abaa abaa

abaababaabaababaabaababa

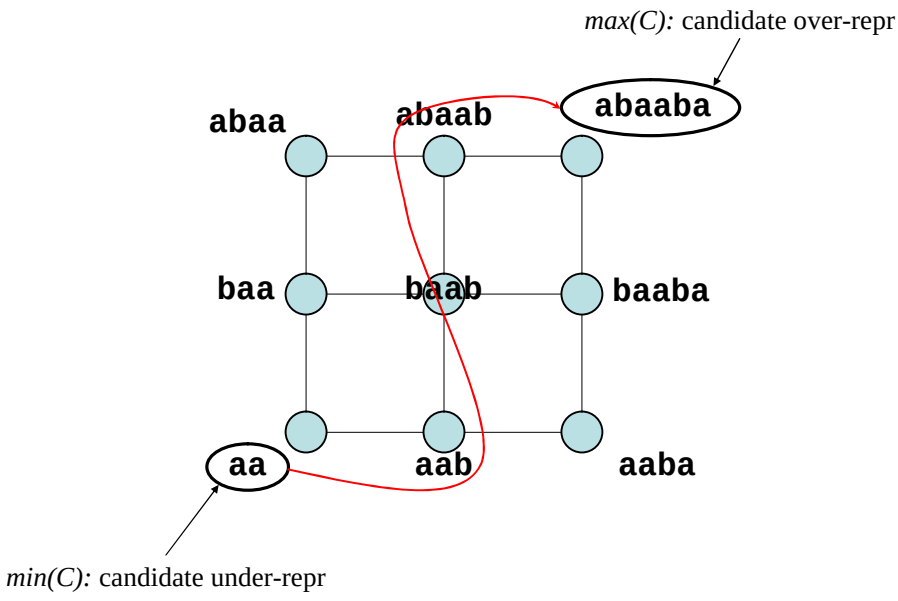
abaab abaab

abaababaabaababaabaababa

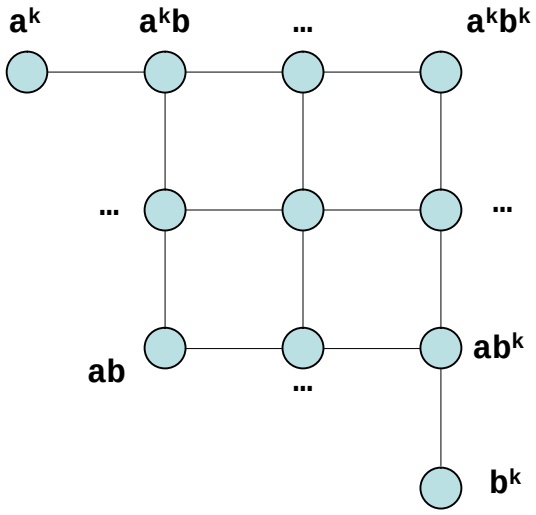
abaaba abaaba

abaababaabaababaababa

abaaba abaaba



$x = a^k b^k$

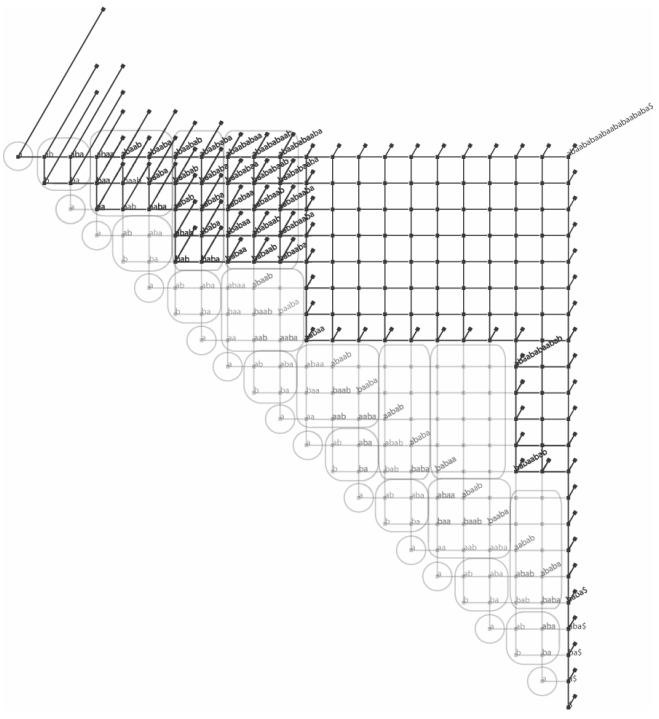


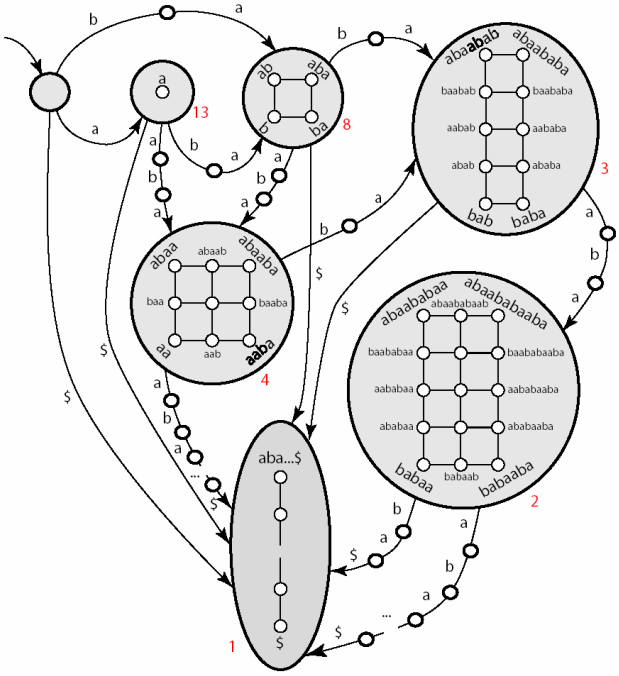
The partition $\{C_1, C_2, \ldots, C_l\}$ of the set of all substrings of x, has to satisfy the following properties, for all $1 \leq i \leq l$,

- $\min(C_i)$ and $\max(C_i)$ are unique
- all w in C_i belong to some $(\min(C_i), \max(C_i))$-path
- all w in C_i have the same count

x = abaababaabaababababa

a (13)	b ba ab aba (8)	babaa babaab babaaba ababaa ababaab ababaaba aababaa aababaab aababaaba baababaa baababaab baababaaba abaababaa abaababaab abaababaaba (2)	…….. …….. …….. …….. …….. …….. …….. …….. …….. …….. …….. …….. …….. …….. …….. (1)
aa aab aaba baa baab baaba abaa abaab abaaba (4)	bab baba abab ababa ababb aababa baabab baababa (3)		





Theorem:

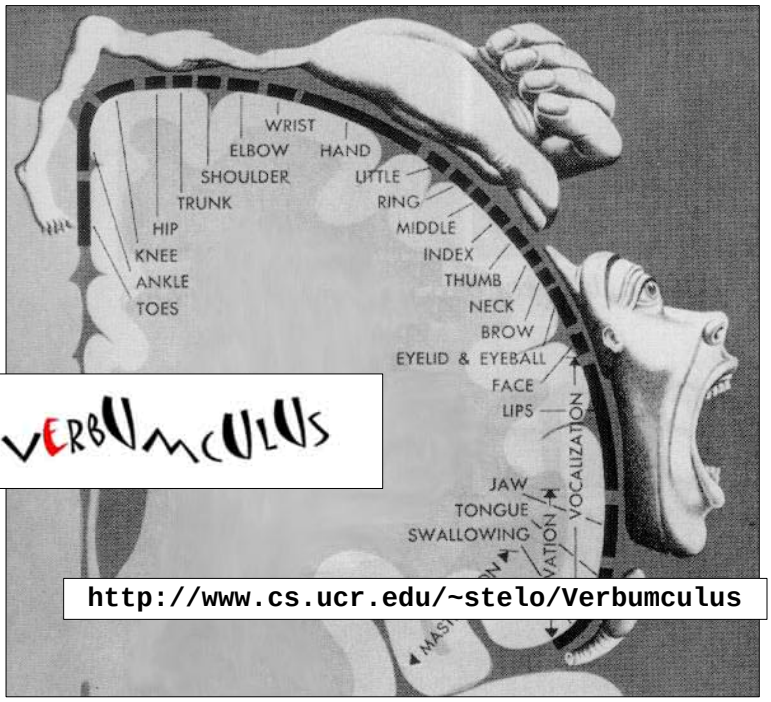
The number of classes is at most $2n$

Computing on the Suffix Tree

- Equivalence classes can be computed in *O(n)* time (by merging isomorphic sub-trees of the suffix tree [ABL, Recomb02])
- Expectations, variances and scores can be computed in *amortized constant time* per node [ABLX, JCB00]

Theorem:

The set of over- and under-represented patterns can be detected in $O(n)$ time and space



Conclusions on Verbumculus

- Pros:
  - exhaustive
  - linear time and space
- Cons:
  - limited to deterministic patterns

Discovering Rigid Patterns

Complexity results

- Li *et al.*, [STOC 99] proved several important theoretical facts
- Many of the problems in pattern discovery turn out to be NP-hard
- For some there is a polynomial time approximation scheme (PTAS)

Consensus Patterns

- Consensus patterns problem: Given a multisequence $\{x_1,x_2,\ldots,x_k\}$ each of length n and an integer m, FIND a string y of length m and substring t_i of length m from each x_i such that $\Sigma_i\ h(y,t_i)$ is minimized

- Theorem [Li *et al.*, 99]: The consensus pattern problem is NP-hard

Closest string

- Closest string problem: given a multisequence $\{x_1,x_2,\ldots,x_k\}$ each of length n, FIND a string y of length n and the minimum d such that $h(y,x_i)\leqslant d$, for all i

- Theorem: The closest string problem is NP-hard

Closest substring

- Closest substring problem: given a multisequence $\{x_1,x_2,\ldots,x_k\}$ each of length n and an integer m, FIND a string y of length m and the minimum d such that for each i there is a substring t_i of x_i of length m satisfying $h(y,t_i) \leqslant d$

- Theorem: The closest substring problem is NP-hard (it is an harder version of Closest string)

NP-hard: what to do?

- Change the problem
  - e.g., “relax” the class of patterns

- Accept the fact that the method may fail to find the optimal patterns
  - Heuristics
  - Randomized algorithms
  - Approximation schemes

Discovering Rigid Patterns

- We report on four recent algorithms

- Teiresias [1998]
- Winnower [2000]
- Projection [2001]
- Weeder [2001]

- (disclaimer: my selection is biased)

Teiresias

Teiresias algorithm

- By Rigoustos and Floratos [Bioinformatics, 1998]
- Server at http://cbcsrv.watson.ibm.com/Tspd.html
- The worst case running time is exponential, but works reasonably fast on average
- A recent improved algorithm runs in polynomial time by reporting only to *irredundant* patterns [Parida *et al.*, 2000]

Teiresias patterns

- Teiresias searches for rigid patterns on the alphabet $\Sigma \cup \{.\}$ where "." is the don't care symbol
- However, there are some constrains on the density of "." that can appear in a pattern

$<L,W>$ patterns

- Definition: Given integers L and W, $L \leqslant W$, y is a $<L,W>$ pattern if
  - y is a string over $\Sigma \cup \{.\}$
  - y starts and ends with a symbol from Σ
  - any substring of y containing exactly L symbols from Σ has to be shorter (or equal) to W

Example of $<3,5>$ patterns

- **`AT..CG..T`** is a $<3,5>$ pattern
- **`AT..CG.T.`** is not a $<3,5>$ pattern, because it ends with "."
- **`AT.C.G..T`** is not a $<3,5>$ pattern, because the substring **`C.G..T`** is 6 characters long

Teiresias

- Definition: A pattern *w* is more *specific* than a pattern *y*, if *w* can be obtained from *y* by changing one or more "." to symbols from Σ, or by appending any sequence of $\Sigma \cup \{.\}$ to the left or to the right of *y*

- Example: given *y* = **AT.CG.T**, the following patterns are more specific then *y*
  ATCCG.T, CAT.CGCT, AT.CG.T.A, T.AT.CGTT.A

Teiresias

- Definition: A pattern *y* is *maximal* with respect to the sequences $\{x_1,x_2,\ldots,x_k\}$ if there exists no pattern *w* which is more specific than *y* and $f(w)=f(y)$

- Given $\{x_1,x_2,\ldots,x_k\}$ and parameters *L*,*W*,*K*, Teiresias reports *all* the *maximal* $<L,W>$ patterns that have at least *K* colors

Teiresias algorithm

- Idea: if *y* is a <*L*,*W*> pattern with at least *K* colors, then its substrings are also <*L*,*W*> patterns with at least *K* colors
- Therefore, Teiresias assembles the maximal patterns from smaller patterns
- Definition: A pattern *y* is *elementary* if is a <*L*,*W*> pattern containing exactly *L* symbols from Σ

Teiresias algorithm

- Teiresias works in two phases
  - Scanning: find all elementary patterns with at least *K* colors; these become the initial set of patterns
  - Convolution: repeatedly extend the patterns by "gluing" them together
- Example: *y* = **AT..CG.T** and *w* = **G.T.A** can be merged to obtain **AT..CG.T.A**

Convolution phase

- For each elementary pattern *y*, try to extend it with all the other elementary patterns
- Any pattern that cannot be extended without losing support can be potentially maximal

Convolution phase

- To speed-up this phase, one wants to avoid the all-against-all comparison
- The authors devise two partial orderings $<_{pf}$ and $<_{sf}$ on the universe of patterns
- Using these orderings to schedule the convolution phase, they guarantee that
  - all patterns are generated
  - a maximal pattern *y* is generated before any non-maximal pattern subsumed by *y*

Partial ordering $<_{pf}$

- Definition: determine whether $y <_{pf} w$ or $w <_{pf} y$ using the following algorithm
  - align y and w such that the leftmost residues are in the same column
  - examine one column after the other (left to right) and stop whenever one column has a residue and the other has a "."
  - if the residue comes from y then $y <_{pf} w$
  - if the residue comes from w then $w <_{pf} y$

Example

- $y =$ **ASD...F**
  $w =$ **SE.ERF.DG**
  $y <_{pf} w$

- $y =$ **ASD...F**
  $w =$ **SE.ERF.DG**
  $w <_{sf} y$

Teiresias algorithm

- Initialize the stack with elementary patterns with support at least K
- Order the stack according to $<_{pf}$ and $<_{sf}$
- Repeat
  - Repeat
    - Try to extend the top pattern to the right with all the others in the prefix-wise ordering
    - If a new pattern is formed with have enough support, it becomes the new top
  - Until the top can no longer be extended to the right
  - Do the same for left extension, using the ordering $<_{sf}$
  - Check the top for maximality, if so pop it and report it
- Until stack is empty

Conclusions on Teiresias

- It can be proved that Teiresias correctly reports all $<L,W>$ maximal patterns
- Pros:
  - provably correct
  - fast on average input
- Cons:
  - exponential time complexity
  - limited to $<L,W>$ patterns

Winnower

Pevzner and Sze, UCSD

Winnower

- Invented by Pevzner and Sze [ISMB 2000]
- Initially designed to solve the *(15,4)*-motif challenge
- Planted *(m,d)*-motif problem:
  - The problem is to determine an *unknown* pattern *y* of length *m* in a set of *k* nucleotide sequences, each of length *n*, and each one containing exactly one occurrence of a string *w* such that $h(y,w)=d$

Winnower

- Pevzner and Sze show that the most popular algorithms (Consensus, GibbsDNA, MEME) fail to solve (most of the times) the *(15,4)*-motif problem *[n=600, k=20]*
- (Note: this comparison is not totally *fair)*
- Why the *(15,4)*-motif problem is difficult?
- Because two strings in the class of the *(15,4)* unknown pattern may differ by as many as 8 positions out of 15, a rather large number

Winnower

- Idea: Search for groups of strings of length *m* such that any two in a group differ at most by *2d* positions
- Remember however that this may not be sufficient

Winnower

- How to find groups of patterns such that given any two elements w_1 and w_2 in the group, $h(w_1,w_2) \leqslant 2d$?
- One could generate *(k choose 2)* multiple alignments to find out all pairs of substrings of length *m* that have at most *2d* mismatches (Consensus [Hertz & Stormo 1999])

Winnower

- Winnower builds a graph *G* in which
  - each vertex corresponds to a distinct string of length *m*
  - two vertices are connected by an edge if the Hamming distance between the corresponding strings is at most *2d*, and the strings do not come from the same sequence (remember that we are guaranteed that there is only one occurrence of the unknown pattern in each sequence)

Graph for the *(15,4)*-problem

- They report that for each “signal”-edge there are about 20,000 spurious-edges
- Finding the signal among the noise is a “daunting task”

Winnower

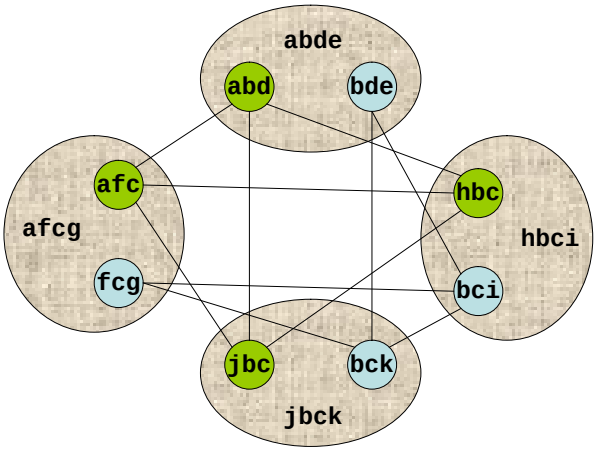
- Winnower searches the graph *G* for *cliques*, which are subsets of vertices totally connected
- But the problem of finding large cliques in graphs is *NP*-complete

Multipartite graphs

- Definition: A graph *G* is *n*-partite if its vertices can be partitioned into *n* sets, such that there is no edge between any two vertices within a set
- Fact: Winnower's graph is *k*-partite

Example

- Given sequences {**abde,afcg,hbci,jbck**} we look for a *(3,1)*-motif



Idea

- Each vertex of the clique has to be in a different partition
- We look for cliques that have exactly one vertex in each partition

Extendable cliques

- Definition: a vertex u is a *neighbor* of a clique $\{v_1,\ldots,v_s\}$ if $\{v_1,\ldots,v_s,u\}$ is also a clique for G, when $s<k$
- Definition: a clique is called *extendable* if it has at least one neighbor which has at least one vertex in every part of the k-partite graph G

Extendable cliques

- Definition: A clique with k vertices, each in a different partition is called *maximal*
- Consider a maximal clique and take a subset of t of its vertices: this subset is an extendable clique
- Idea: remove edges that do not belong to extendable cliques

Extendable cliques

Fact: For any clique of size k there are $\binom{k}{t}$ extendable cliques with t vertices

Fact: Any edge belonging to a clique with k vertices is member of at least $\binom{k-2}{t-2}$ extendable cliques of size t

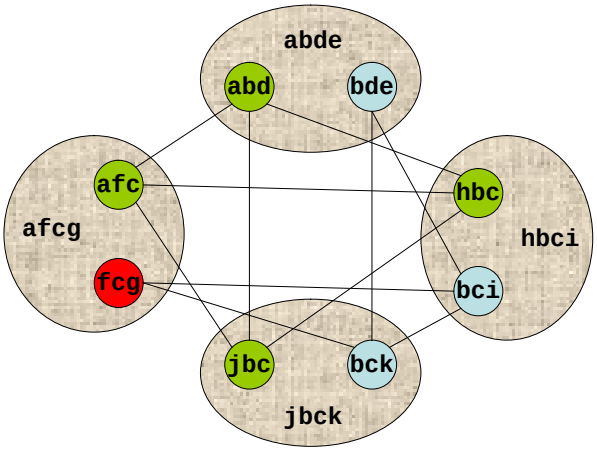
Idea

An edge that is not member of at least $\binom{k-2}{t-2}$ expandable cliques of size t cannot be part of a maximal clique and therefore it can be removed

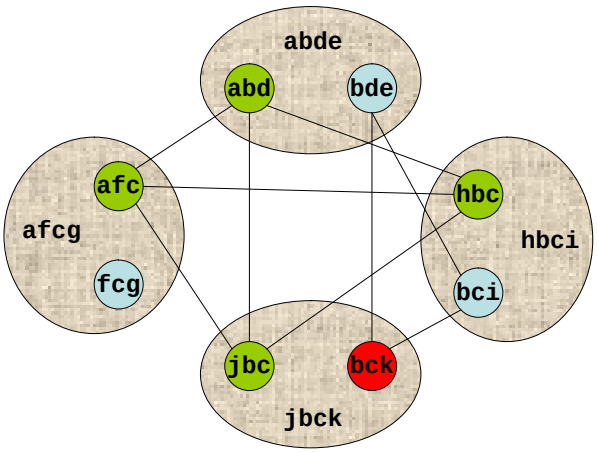
t=1

- For *t=1*, each vertex is a clique
  - it is extendable if it is connected to at least one vertex in each partition
- Delete all edges corresponding to vertices that do not have a neighbor in each partition
- Iterate

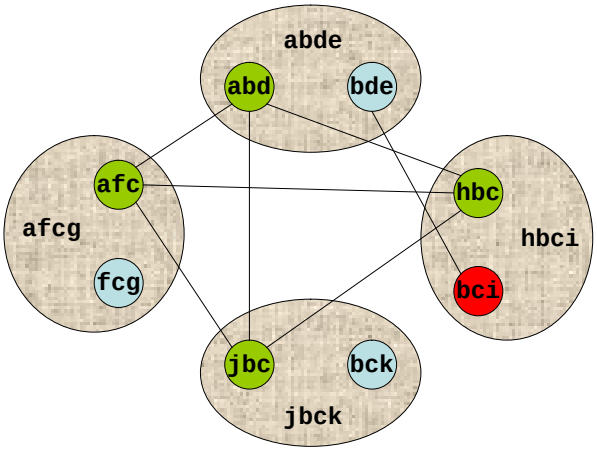
Example



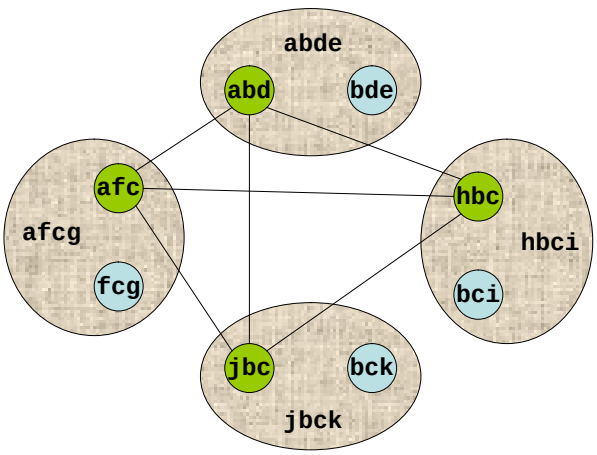
Example



Example



Example



t=2

- For *t=2*, each pair of vertices *u,v* such that there is an edge *(u,v)* is a clique
  - it is extendable if there is vertex *z* in each of the other *k-2* partitions such that *(u,v,z)* is a cycle of length 3
  - each edge should belong to at least *(k-2 choose t-2)=(n-2 choose 0)=1* clique of size 2

t>2

- For *t=3*, Winnower removes edges that belong to less than *k-2* extendable cliques of size *3*
- For *t=4*, Winnower remove edges that belong to less than *(k-2)(k-1)/2* extendable cliques of size *4*
- …

Remarks on Winnower

- Pros:
  - more effective than Meme, Consensus and GibbsDNA for the (15,4) problem
- Cons:
  - randomized
  - time-complexity can be very high (e.g., for $t=3$ is $O(n^4)$)
  - need to know m and d in advance
  - assume exactly one occurrence per sequence

Projection

Random Projection algorithm

- Proposed by Buhler and Tompa [Recomb 2001]
- The algorithm was initially designed to solve the *(m,d)*-motif planted problem

Analysis on *(m,d)*-motif problem

Suppose A,C,T,G have probability $1/4$. Then the probability that a pattern of size m occurs at a given position is $p_{(0)} = (1/4)^m$. If we allow up to one mismatch, the probability becomes $p_{(1)} = p_{(0)} + m(3/4)(1/4)^{m-1}$. If we allow at most two, it becomes $p_{(2)} = p_{(1)} + \frac{m(m-1)}{2}(3/4)^2(1/4)^{m-2}$. In general, if we allow up to d mismatches, $p_{(d)} = \sum_{i=0}^{d} \binom{m}{i} \left(\frac{3}{4}\right)^i \left(\frac{1}{4}\right)^{m-i}$.

Analysis on *(m,d)*-motif problem

If Z is the r.v. for the number of occurrences,

then $P(Z>0) = 1 - P(Z=0) = 1 - (1 - p_{(d)})^{n-m+1}$

If we have k sequences, we get that the probability that a particular y occurs at least once in each sequence is $\left(1 - (1 - p_{(d)})^{n-m+1}\right)^k$.

Therefore, the expected number of patterns is

$E(n,m,k,d) \equiv 4^m \left(1 - (1 - p_{(d)})^{n-m+1}\right)^k$.

Stats of spurious *(m,d)*-motifs in simulated data *(k=20,n=600)*

m	*d*	*E(600,m,20,d)*	*E(600,m+1,20,d)*	apc	Correct	Spurious	19/20	*iter*
9	2	1.6	6.1×10^{-8}	0.28	11	5	4	1483
11	3	4.7	3.2×10^{-7}	0.026	1	13	6	2443
13	4	5.2	4.2×10^{-7}	0.062	2	15	3	4178
15	5	2.8	2.3×10^{-7}	0.018	0	7	13	6495
17	6	0.88	7.1×10^{-8}	0.022	0	8	12	9272

Bottom-line: the *(9,2)-, (11,3)-, (13,4)-, (15,5)-* and *(17,6)*-motif problems are probably impossible to solve

Random Projections

- Idea: select *t* random positions and for each substring of length *m* of the text hash its selected positions into a table
- Hopefully, the cell corresponding to the planted motif will be the one with the highest count

Random Projection algorithm

- Parameters *(m,d), n, k, s,* possibly *i*
- Set $t < m-d$ and $4^t > k(n-m+1)$
- Build a table with all substrings of length *m*
- Repeat *i* times
  - Select randomly *t* positions
  - Repeat for all substrings in the table
    - Increase the count of the cell indexed by the *t* positions
- Select all cells with count $\geqslant s$

Random Projection algorithm

- We want $t < m-d$ because we want to sample from the "non-varying" positions
- The number of iterations i can be estimated from m, d and t

Random Projection algorithm

- Since we are hashing $k(n-m+1)$ substrings of size m into 4^t buckets, if $4^t > k(n-m+1)$ each bucket will contain on average less than one substring (set $s=1$)
- The constrain is designed to filter out the noise
- The bucket corresponding to the planted motif is expected to contain *more* motif instances than those produced by a random sequence

Random Projection algorithm

- If the constrain $4^t > k(n-m+1)$ cannot be enforced, the authors suggest to set
  $t = m-d-1$ and the threshold
  $s = 2\,[k(n-m+1)/4^t]$ (twice the average bucket size)

Motif refinement

- The algorithm will try to recover the unknown motif from each cell having at least *s* elements
- The primary tool for motif refinement is expectation maximization (EM)

Experiments

- Projection can handle the *(15,4)- (14,4)- (16,5)-* and *(18,6)*-motif problem *(k=20, n=600)*
- Winnower fails the *(14,4)- (16,5)-* and *(18,6)*-motif problem

Results

m	*d*	Gibbs	WINNOWER	SP-STAR	PROJECTION	Correct	iter
10	2	0.20	0.78	0.56	0.82	20	72
11	2	0.68	0.90	0.84	0.91	20	16
12	3	0.03	0.75	0.33	0.81	20	259
13	3	0.60	0.92	0.92	0.92	20	62
14	4	0.02	0.02	0.20	0.77	19	647
15	4	0.19	0.92	0.73	0.93	20	172
16	5	0.02	0.03	0.04	0.70	16	1292
17	5	0.28	0.03	0.69	0.93	19	378
18	6	0.03	0.03	0.03	0.74	16	2217
19	6	0.05	0.03	0.40	0.96	20	711

k=20, n=600, winnower *(t=2),* projection *(t=7,s=4, 20* random instances*)*

Remarks about Projection

- Pros:
  - fast and effective
- Cons:
  - need to know *m* and *d* in advance
  - randomized

Weeder

Weeder

- Proposed by Pavesi, Mauri and Pesole [ISMB 2001]
- Draw ideas from PRATT by [Jonassen95, Jonassen97] and [Sagot98]
- It is an exhaustive approach for a particular class of rigid patterns

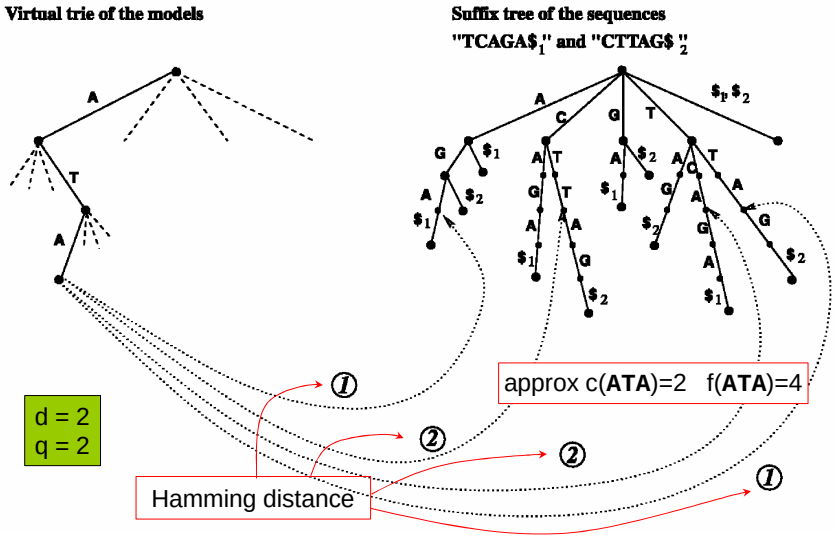
Exhaustive approach

- Suppose that you want to spell out all possible *(m,d)* rigid patterns that has at support least *q*
- One way to do it, is to use a (generalized) suffix tree [Sagot 98]

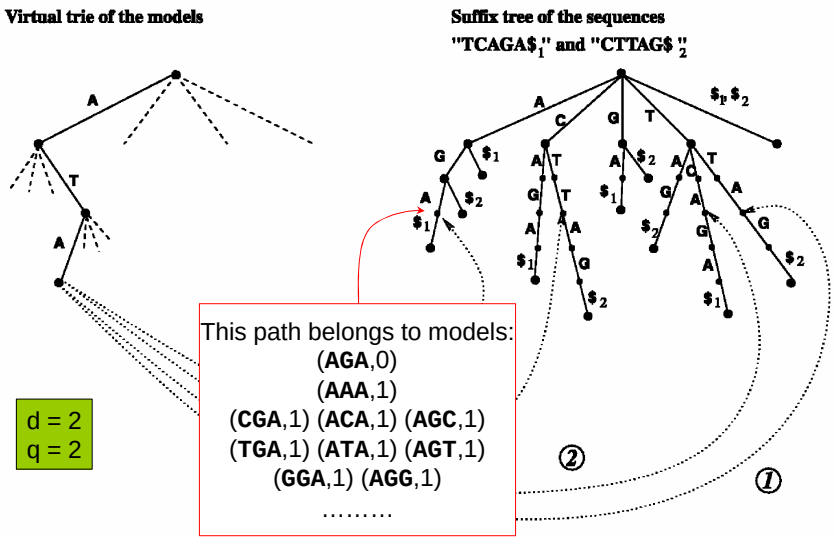
Idea [Sagot 98]

- Any deterministic pattern (substring) *w* corresponds to a path in the tree ending in a node *u*, called the *locus* of *w* – the number of leaves in the subtree rooted at *u* gives the support
- Any model (rigid pattern) corresponds to a set of paths in the tree ending in nodes $\{u_1,u_2,\ldots,u_l\}$ – the total number of leaves in the subtrees rooted at $\{u_1,u_2,\ldots,u_l\}$ gives the support

Example



Example



Exhaustive approach [Sagot 98]

- Start with all paths of length *d* with enough support (they represent valid models)
- At each path-extension keep track of the mismatches and the support
  - if the number of mismatches has not been reached the model will be extended by the symbols in Σ (therefore the number of models will be scaled up by a factor $|\Sigma|$)
  - otherwise we are allowed just to follow the arcs

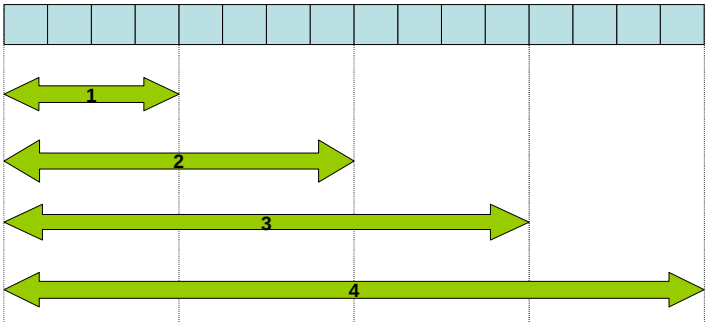
Time complexity [Sagot 98]

- Finding all the models with support=occurrences in a single sequence takes $O(n\ N(m,d)) = O(n\ m^d\ |\Sigma|^d)$
- Finding all the models with support=colors in a multisequence takes $O(n\ k^2\ N(m,d)) = O(n\ k^2\ m^d\ |\Sigma|^d)$
- Note that the complexity is exponential (with *d)*

Weeder

- Pavesi *et al.*, implemented the algorithm by Sagot but it was running too slow, and they decided to change the class of patterns
- Weeder is designed to find rigid patterns which have an amount of mismatches proportional to their length (the same constrain applies also to all their prefixes)

Example ε =0.25



Time complexity

- By restricting the number of mismatches to εm, the time complexity becomes $O(n\, k \lceil 1/\varepsilon \rceil^{\varepsilon m}\, |\Sigma|^{\varepsilon m})$

The *(15,4)*-motif challenge … again

- Since the restriction on the density of the mismatches, the authors report that Weeder has probability *0.6* to catch the motif in ONE sequence
- Then, the probability of Weeded to get the motif in all the 20 sequence is almost zero
- On the other hand, running the Sagot's version is too time-consuming

Idea

- Split the set of sequence into two halves
- Run Weeder on each of the two sets requiring support *k/4* (instead of *k/2)*
- The probability that the *(15,4)*-motif will be in either subset is 0.98
- The pool of model candidates is then processed with Sagot's algorithm

Remarks about Weeder

- Pros:
  - Possibly exhaustive (if using Sagot's algorithm)
  - The relative error rate ε may be more meaningful than d and allows one not to specify in advance m
- Cons:
  - Very slow if run exhaustively - it cannot be considered exhaustive in practice

Discovering Profiles

Discovering Profiles

- If one assumes the unknown profile to have been generated by a sequence of independent r.v.s then the observed frequency of letters in the columns of the profile are the ML estimates of the distributions of the r.v.s
- Unfortunately we do not know the positions of the profile in the multisequence

Gibbs sampler

Gibbs sampling

- Proposed by Lawrence, *et al.*, [Science, 1993]
- Web servers at
  http://bayesweb.wadsworth.org/gibbs/gibbs.html and
  http://argon.cshl.org/ioschikz/gibbsDNA/
- Input: multisequence $\{x_1,x_2,\ldots,x_k\}$
  pattern length m
- Output: a matrix profile $q_{i,b}$, $b \hat{I} \Sigma$, $1 \leqslant i \leqslant m$, and positions s_j, $1 \leqslant j \leqslant k$, of the profile in the k sequences

Gibbs sampling

- The algorithm maintains the background distribution $p_A,\ldots,p_T$ of the symbols not described by the profiles
- $P(y)$ is the probability of y based on the background distribution p_b, $b \hat{I} \Sigma$
- $Q(y)$ is the probability of y based on the profile $q_{i,b}$, $1 \leqslant i \leqslant m$, $b \hat{I} \Sigma$

Gibbs sampling

- Idea: the profile is obtained by locating the positions which maximizes *Q(y)/P(y);* once the positions are obtained a new, more accurate, version of the profile can be obtained

- Initialize the initial positions s_j randomly

Gibbs sampling

Gibbs sampler iterates 1), 2) until convergence

1) Predictive update step: randomly choose one of the k sequences, say r. The matrix profile $q_{i,b}$ and the background frequencies p_b are recomputed from the current positions s_j in all sequences excluding r
2) Sampling step: assign a weight $z(y)=Q(y)/P(y)$ to each substring y of length m. Select randomly a substring y with probability $z(y)/\sum_y z(y)$, and then update s_j

Gibbs sampling

- The more accurate the pattern description in step 1), the more accurate the determination of its position in step 2), and vice versa
- Once some correct positions have been selected by chance, $q_{i,b}$ begins to reflect, albeit imperfectly, the unknown pattern
- This process tends to recruit further correct positions which in turn improve the discriminating power of the evolving pattern

Gibbs sampling

- How to update the matrix profile $q_{i,b}$ and the background frequencies p_b?
- We set $q_{i,b}=(f^i(b)+d_b)/(k-1+\sum_c d_c)$ where $f^i(b)$ is the number of times we observe symbol b in the position i of the profile (currently placed at position s_j), except for sequence r (d_b are pseudo-counts)
- We set the background probabilities $p_b=f(b)/\sum_c f(c)$ for all symbols in positions not covered by the profile

Phase shift problem

- Suppose that the “strongest” pattern begin, for example, at position *7, 19, 8, 23, …*
- If Gibbs happens to choose $s_1=9$, $s_2=21$ it will most likely choose $s_3=10$ and $s_4=25$
- The algorithm can get stuck in local maxima, which are the shifted form of the optimal pattern

Phase shift problem

- The problem can be alleviated by adding a step in which the current set of positions are compared with sets of shifted left and right positions, up to a certain number of symbols
- Probability ratios may be calculated for all positions, and a random selection is made with respect to the appropriate weight

Gibbs sampling

- It can be generalized to:
- Find also the length of pattern *m*
- Find a set of matrix profiles, instead of one

Gibbs sampling

- Since Gibbs sampler is an heuristic rather than a rigorous optimization procedure, one cannot guarantee the optimality of the result
- It is a good practice to run the algorithm several times from different random initial positions

Gibbs sampling vs. EM

- Although EM and Gibbs are built on common statistical foundation, the authors claim that Gibbs outperforms EM both in term of time complexity and performance

- *"EM is deterministic and tends to get trapped by local optima which are avoided by Gibbs … HMMs permit arbitrary gaps … have greater flexibility, but suffer the same penalties …"*

Expectation Maximization and MEME

Expectation maximization

- EM was designed by Dempster, Laird, Rubin [1977]
- EM is a family of algorithms for maximum likelihood estimation of parameters with "missing data"

EM, when?

- When we want to find the maximum likelihood estimate of the parameters of a model and
  - data is incomplete, or
  - the optimization of the maximum likelihood function is analytically intractable but the likelihood function can be simplified by assuming the existence of additional, *missing*, parameters value

Expectation maximization

- EM approaches the problem of missing information by iteratively solving a sequence of problems in which expected information is substituted for missing information

Expectation maximization

- All EM algorithms consists of two steps:

1) the expectation step (E-step)

2) the maximization step (M-step)

- The expectation step is with respect to the unknown underlying variables, using the current estimate of the parameters and conditioned upon the observation

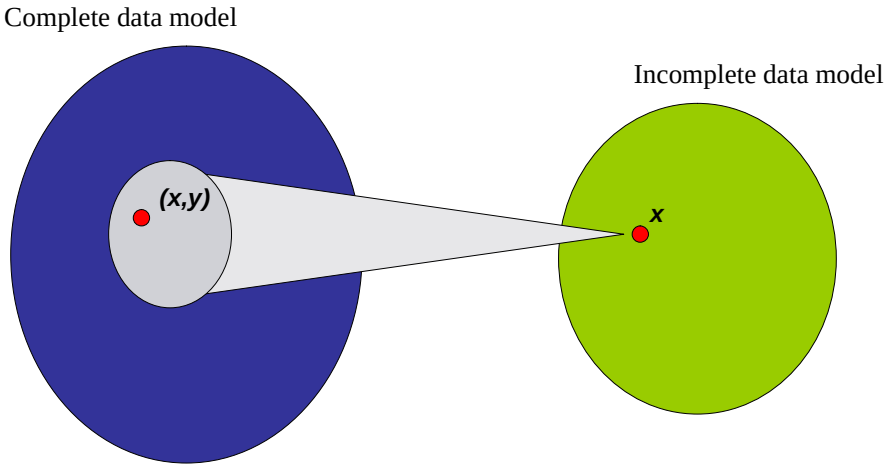
Expectation maximization

- The maximization step provides a new estimate of the parameters
- $\theta^1 \blacktriangleright \theta^2 \blacktriangleright \theta^3 \blacktriangleright \ldots \blacktriangleright \theta^t \blacktriangleright \theta^{t+1} \blacktriangleright \ldots$
- The two steps are iterated until convergence

General framework for EM

- Suppose we want to find the parameters θ of a model (*training*)
- We observe *x* (*training set*)
- The probability of *x* under θ is also determined by the missing data *y*

Incomplete data model



An occurrence of *(x,y)* implies an occurrence of *x*, however only *x* can be observed. This observation reveals the subset *{(x,y), for all y}*

Expectation maximization

- Example: For HMMs, *x* is the sequence we want to learn from, θ is the transition and emission probabilities, *y* is the path through the model
- Example: In the case of Random Projections, *x* are the subsequences corresponding to a cell with count higher than the threshold, θ are the parameters of a representation of the *(m,d)* pattern, *y* are all the missing positions

MEME

- Proposed by Bailey and Elkan [Machine Learning J., 1995]
- "Multiple EM for Motif Elicitation" (MEME) is an improved version of the expectation maximization approach by Lawrence and Reilly [Proteins, 1990] (see appendix)
- Designed to discover profiles (no gaps)
- Server at http://meme.sdsc.edu/meme/

MEME

- There are three main differences w.r.t. Lawrence *et al.:*

1) the initial profiles are not chosen randomly, but they are substrings which actually occur in the sequences
2) the assumption that there is only one occurrence of the motif is dropped
3) once a profile has been found, it is reported, and the iterative process continues

Using substring as starting points

- Idea: substrings actually occurring in sequence are better starting points than random choices
- Each substring is converted into a profile
- Assigning 1.0 to the occurring symbol and 0.0 to the others is a bad choice, because EM cannot move from this
- The authors arbitrarily assign probability 0.5 to the symbol and 0.5/3 for the other three

Using substring as starting points

- It would be too expensive to run EM until convergence from each substring
- It turns out that this is not necessary
- EM converges very quickly from profiles obtained from substrings, and the best starting point can be found running only one iteration

MEME algorithm

- Repeat
  - For each substring y in $\{x_1,x_2,\ldots,x_k\}$ do
    - Run one EM iteration with profile computed from y
    - Choose the profile q with highest likelihood
    - Run EM until convergence starting from q
    - Report the profile q
    - Erase the occurrences of q from dataset
- Until max number of iterations is reached

Dealing with multiple occurrences

- MEME allows to drop the “one-per-sequence” assumption
- The basic idea is to require the user to supply an estimated number of occurrences of the unknown profile and use that to normalize the estimation process of the EM algorithm
- The authors claim that the exact value of the number of occurrences is not critical

Finding multiple profiles

- MEME does not stop after finding the most likely profile
- Once a profile is found and reported, it is "probabilistically erased" by changing some position-dependent weight
- The process continues until a number of predetermined motifs have been found
- (see appendix for mega-prior heuristic)

THE END

Latest version of the slides at http://www.cs.ucr.edu/~stelo/ismb02/
Check out also http://www.cs.ucr.edu/~stelo/cs260/