An Efficient Algorithm for the Extended (*l*,*d*)-Motif Problem With Unknown Number of Binding Sites*

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Abstract

Finding common patterns, or motifs, from a set of DNA sequences is an important problem in molecular biology. Most motif-discovering algorithms/software require the length of the motif as input. Motivated by the fact that the motif's length is usually unknown in practice, Styczynski et al. introduced the Extended (l,d)-Motif Problem (EMP), where the motif's length is not an input parameter. Unfortunately, the algorithm given by Styczynski et al. to solve EMP can take an unacceptably long time to run, e.g. over 3 months to discover a length-14 motif.

This paper makes two main contributions. First, we eliminate another input parameter from EMP: the minimum number of binding sites in the DNA sequences. Fewer input parameters not only reduces the burden of the user, but also may give more realistic/robust results since restrictions on length or on the number of binding sites make little sense when the best motif may not be the longest nor have the largest number of binding sites. Second, we develop an efficient algorithm to solve our redefined problem. The algorithm is also a fast solution for EMP (without any sacrifice to accuracy) making EMP practical.

1. Introduction

A *gene* is a segment of DNA that is the blueprint for protein. Genes seldom work alone. In most cases, genes cooperate to produce different proteins to provide a particular function. Understanding how the *gene regulatory network* works is important in molecular biology.

In order to start the decoding process (*gene expression*), a molecule called *transcription factor* will bind to a short region (*binding site*) preceding the gene. One kind of transcription factor can bind to the binding sites of several genes to cause these genes to co-express. These binding sites have similar patterns called *motifs*. Finding motifs from a set of DNA sequences is a critical step for understanding the gene regulatory network.

In order to discover motifs, we must first have a model to represent a motif. There are three common models: matrix representation, regular grammar representation and string representation.

In the matrix representation model, motifs are represented by *position weight matrices* (PWMs) or *position specific scoring matrices* (PSSMs). Both PWMs and PSSMs incorporate probabilities and thus may represent motifs in real biological data better than the other representations. However, since the solution space for PWMs and PSSMs is infinite in size, algorithms generally either produce a sub-optimal motif matrix (e.g. algorithms [1, 5, 7, 11] relying on local search), or take too long to run when the motif is longer than 10 bp (e.g. algorithms [9] relying by partitioning).

Some algorithms [15, 20] use regular grammars to represent motifs. They assume that all binding sites are patterns satisfying a set of rules, which cannot be satisfied by sequences in non-binding regions. Typically, these algorithms find the optimal grammar from a restricted class of regular grammars by exhaustion and hence the running time for these algorithms tends to be long.

Algorithms using string representation [2-4, 6, 8, 10, 13, 16-24] assume all binding sites are variants of the motif. Pevzner and Sze [17] give a precise definition of motif discovery problem based on string representation.

Planted (*l*,*d*)-Motif Problem (PMP): Suppose there is a fixed but unknown nucleotide sequence M (the motif) of length l. Given t length-n sequences, each of which contains exactly one planted *variant* (binding site) of M, we want to determine M without knowing the positions of the planted variants. A *variant* is a length-l string derivable from M with exactly d point substitutions. □

Many algorithms have been developed to solve PMP. Some work efficiently when l is small (≤ 20) [3, 4]. However, PMP is an inadequate model of reality. There are three main weaknesses in PMP. First, biologists seldom get a set of sequences where each contains exactly

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one planted variant. Due to experimental noise and error, they usually get a set of sequences, some of which contain no variants, some exactly one and some more than one. Second, biologists usually do not know the motif's exact length l; at best they only know the range for the length. Third, the Hamming distance between each variant and the motif may not be exactly d.

Consequently, researchers [4, 19] have modified the PMP to better model reality. Modifications include allowing each sequence to contain any number of variants and allowing the Hamming distance between a variant and the motif M to be at most d (instead of exactly d).

Based on the assumption that it is easier to estimate the ratio d/l than l, Styczynski et al. [22] defined the Extended (l,d)-Motif Problem as follows.

Extended (l,d)-Motif Problem (EMP): Suppose there is a fixed but unknown nucleotide sequence M of length L. Given t length-n input sequences, containing a total of at least k planted *(l,d)-variants* of M where $l \leq L$, we want to determine M without knowing the positions of the planted (l,d)-variants in the input sequences and length L. A *(l,d)-variant* is a length-L string derivable from M with at most d point substitutions over any window of l nucleotides, where $l \leq L$. □

In practice, many motifs are similar in the sense that the positions of the (l,d)-variants for these motifs are very close. Therefore, Styczynski et al. [22] used a "maximal motif" concept to represent a set of similar motifs. They also developed an algorithm to find all maximal motifs given the input parameters l, d and k.

Definition of maximal motif: A sequence M is a *maximal motif* if it satisfies the following properties:

1) The length of M is at least l.
2) M has at least k (l,d)-variants in the input sequences.
3) The length of M cannot be increased without producing a motif with fewer (l,d)-variants in the input sequences.
4) The positions of all (l,d)-variants of M cannot start earlier without producing a motif with fewer (l,d)-variants in the input sequences.

Example of maximal motif: We are given the following sequences:

```
    0123456789
S1: TACAGTCGGTGC...
S2: GCCAGTCGGCTG...
S3: CGGAGTCGCGAC...
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Suppose we want to solve EMP with $l = 7$, $d = 1$ and $k = 2$. We may discover four similar motifs M_1, M_2, M_3 and M_4:

```
M1:  GAGTCGG
M2:  GAGTCGGG
M3: GCAGTCGC
M4: GCAGTCGCC
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M_1 has 3 (7,1)-variants at positions $S_i[2\ldots8]$, M_2 at $S_i[2\ldots9]$ and M_3 at $S_i[1\ldots8]$, for $i = 1, 2, 3$. M_4 has 2 (7,1)-variants at positions $S_j[1\ldots9]$ ($j = 2, 3$). However, only M_3 and M_4 are maximal motifs.

M_1 is not a maximal motif because its length can be increased to form motif M_2 with the same number of (7,1)-variants (Property 3 is not satisfied). M_2 is also not a maximal motif because the positions of its (7,1)-variants $S_i[2\ldots9]$ can start earlier at $S_i[1\ldots8]$ to form motif M_3 having the same number of (7,1)-variants (Property 4 is not satisfied). Although M_3 can be extended to form motif M_4, M_3 has 3 (7,1)-variants while M_4 has only 2. Thus, both M_3 and M_4 are maximal motifs. □

EMP is a better model than PMP. However, it is difficult to guess the minimum number of binding sites k in the data set. If the chosen k is too large, we may miss the planted motif. If k is too small, there will be a huge number of outputs (random noise) and we have no idea which one is the planted motif.

Moreover, we have no idea how to compare two maximal motifs: given a short motif with many (l,d)-variants and a longer motif with less (l,d)-variants, we cannot determine which one is more likely to be the planted motif.

Another problem is that although Styczynski et al.'s algorithm for EMP does not miss any maximal motifs, the running time of the algorithm is far too long to be useful in practice. For example, when $t = 20$, $n = 600$, $l = 14$, $d = 4$, the running time of their algorithm takes more than 3 months.

In this paper, we make two main contributions. First, we propose a measurement for comparing motifs of different lengths and numbers of (l,d)-variants in the input sequences by calculating the expected number of random strings having similar properties. Based on this measurement, we modify EMP by eliminating k, the minimum number of (l,d)-variants, as input thus introducing the Further Extended (l,d)-Motif Problem (FEMP). Secondly, we introduce Algorithm exVote, which runs faster than the algorithm proposed by Styczynski et al., to solve EMP as well as FEMP.

This paper is organized as follows. Section 2 describes the measurement for comparing motifs and how to determine the optimal k automatically. Algorithm exVote is described in Section 3. Experimental results on both simulated data and real data are discussed in Section 4, followed by a conclusion in Section 5.

2. Comparing Different Motifs

Given two motifs with different lengths and different number of (l,d)-variants, how can we determine which one is more likely to be the planted motif? In order to answer this question, we should know which motif is more likely be an artifact occurs by random. We calculate the expected number of random sequences with properties similar to each of these two motifs. The motif with fewer random sequences having similar properties is more likely to be the planted motif. Similar analyses have also been used for PMP [3] and the motif problem in matrix representation [5].

2.1. Expected number of random sequences

Assume we are given t length-n random sequences generated according to a particular probability distribution of 'A', 'C', 'G' and 'T' and σ be an arbitrary length-L substring in one of the sequences. Let M be another random sequence with equal occurrence probabilities of 'A', 'C', 'G' and 'T'. The probability that σ is a (l,d)-variant of M is

$$p(L,l,d) = \frac{N(L,l,d)}{4^L}$$

where N(L,l,d) is the number of (l,d)-variants of a length-L sequence. The method for calculation of N(L,l,d) is given in the Appendix. Note that N(L,l,d) can be computed once and stored in a table for future use by the algorithm.

Given t length-n input sequences, the probability that a length-L sequence M has exactly i (l,d)-variants in the input sequences is

$$\binom{t(n-L+1)}{i} p(L,l,d)^i \left(1-p(L,l,d)\right)^{t(n-L+1)-i}$$

By summing up the probability that M has exactly i (l,d)-variants for all $i \geq k$, we can get the probability that M has at least k (l,d)-variants in the t length-n input sequences.

$$P(L,k,l,d) = \sum_{i=k}^{t(n-L+1)} \binom{t(n-L+1)}{i} p(L,l,d)^i \left(1-p(L,l,d)\right)^{t(n-L+1)-i}$$

Since there are 4^L possible length-L sequences, the expected number of length-L sequences having at least k (l,d)-variants in t length-n input sequences is approximately

$$E(L,k,l,d) = 4^L P(L,k,l,d)$$

This formula is only an approximation because the probability of a length-L sequence having at least k (l,d)-variants is not mutually independent of the probability of another length-L sequence having similar properties. However, experiments in Section 4 show that the formula is a good approximation.

If E(L,k,l,d) >> 1, there are many random length-L sequences having similar properties as the planted motif and it would be difficult to identify this planted motif from this set of random sequences. If E(L,k,l,d) << 1, any length-L sequence having at least k (l,d)-variants in the t length-n sequences is unlikely to be random noise and is probably the planted motif.

2.2. Further Extended (l,d)-Motif Problem

When we are given two motifs M_1 and M_2 of length L_1 and L_2 and t length-n input sequences having k_1 and k_2 (l,d)-variants of M_1 and M_2 in respectively, M_1 is more likely to be the planted motif if E(L_1,k_1,l,d) < E(L_2,k_2,l,d). However, when both E(L_1,k_1,l,d) and E(L_2,k_2,l,d) are sufficiently small, say 10^{-5}, even when one is larger than the other, we can say both M_1 and M_2 are planted motifs with confidence.

Instead of discovering the longest motif or the motif with the largest number of (l,d)-variants, we should find those motifs with small expected numbers of random sequences with similar properties. Based on this idea, we modify EMP as follows:

Further Extended (l,d)-Motif Problem (FEMP): Suppose there is a fixed but unknown nucleotide sequence M of length L. Given t length-n sequences containing an unknown but much more than expected number of planted (l,d)-variants of M, we want to determine M and the positions of the planted (l,d)-variants with knowledge of l and d only. □

3. Algorithm

Styczynski et al. [22] developed an algorithm to discover all maximal motifs for EMP. The main idea of their algorithm is based on an observation that if a length-L sequence M has at least k (l,d)-variants in the input sequences, each length-l' substring of M with $l \leq l' < L$ should have at least k (l,d)-variants in the input sequences too. Their algorithm first discovers all length-l motifs with at least k (l,d)-variants in the input sequences (*initial step*) and then merges the length-l (l,d)-variants of these motifs to form the length-(l+1) (l,d)-variants if any two length-l (l,d)-variants overlap at l-1 consecutive positions. A length-(l+1) motif will be formed if it has at least k length-(l+1) (l,d)-variants. This merging step will

be repeated again and again to form longer (l,d)-variants and longer motifs.

Although this algorithm guarantees that no maximal motif is missing, the running time is very long. This is especially so when the Hamming distance d is large. Note that even when E(L,k,l,d) is very small, E(l',k,l,d) can be a very large number when l' is slightly larger than l (Figures 2 and 3). As $(\mathrm{E}(l',k,l,d))^2$ pairs of length-l' motifs have to be studied to form length-(l'+1) motifs, the merging step, which needs to consider

$$\sum_{l'=l}^{L-1}\left(\mathrm{E}(l',k,l,d)\right)^2$$

pairs of length-l' motifs in total, might take a very long time.

As for the initial step, similar to [17], Styczynski et al. reduced the problem of finding all length-l motifs to the clique searching problem. Since the Hamming distance between the motif and its (l,d)-variant is at most d, the Hamming distance between two (l,d)-variants of a particular length-l motif is at most 2d. Consider a graph G where each length-l substring in the input sequences is represented by a vertex and there is an edge joining two vertices if and only if the Hamming distance between the two corresponding length-l substrings is at most 2d. Since a length-l maximal motif (a length-l substring of M) has at least k (l,d)-variants in the input sequences, these (l,d)-variants form a maximal clique of size k or more in the graph. By finding all maximal cliques of size at least k, they can discover all length-l motifs and their corresponding (l,d)-variants (binding sites) in the input sequences.

Graph G has $O(nt)$ vertices and the expected degree of each vertex is $O(ntp)$ where p is the probability that the Hamming distance between the two arbitrary length-l sequences is at most 2d. Assuming each nucleotide is generated independently according to some background probability distribution, p has minimum value when all nucleotides are equally probable in the sequences. The minimum value of p can be calculated as follows:

$$p=\sum_{i=0}^{2d}\binom{l}{i}\left(\frac{3}{4}\right)^i\left(\frac{1}{4}\right)^{l-i}$$

Although clique searching is NP-hard, when d is small, p tends to $1/4^l$ and the graph is sparse. Finding all maximal cliques of size at least k for a sparse graph is still tractable. However, when d increases, the number of edges increases and the graph G becomes dense. For example, when l = 14 and d = 4, the value of p is 0.11 which means that the expected degree of each vertex is 0.11(nt) and the number of edges in G is $O((nt)^2)$. To our best knowledge, the fastest algorithm [14] for finding all maximal cliques in a dense graph is $O((nt)^{2.376})$ per output. Since there are $O((nt)^k)$ maximal cliques in graph G in the worst case, the running time of Styczynski et al.'s algorithm can be $O((nt)^{k+2.376})$, which may be prohibitively long.

3.1. Algorithm exVote

In order to solve EMP (and FEMP), we propose Algorithm exVote, which uses a Voting approach to find all length-l or longer motifs directly. The Voting approach was first introduced by Chin and Leung [4] for solving PMP. To our best knowledge, it is the fastest algorithm for solving PMP. Here we modify the Voting algorithm to solve EMP.

The Voting algorithm [4] is based on a simple idea that if a substring σ is a variant of a motif M, M is also a variant of σ. In order to solve PMP, each length-l substring in the input sequences gives one vote to each of its variants (length-l sequences). Under the restriction that each length-l sequence gets at most one vote from each sequence, the motif should get exactly t votes.

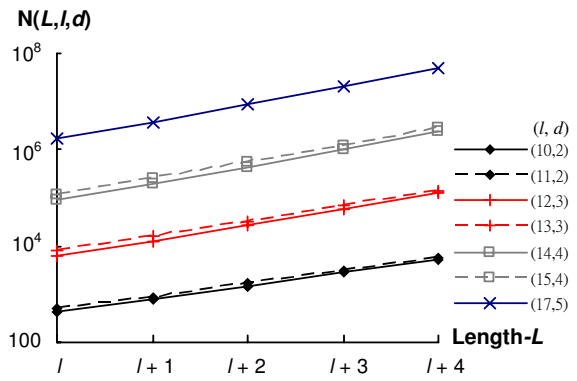
Since EMP allows any number of (l,d)-variants, Algorithm exVote gives one vote to each of the (l,d)-variants of a length-L substring in the input sequences. Those length-L sequences receiving at least k votes are motif candidates. The time complexity of Algorithm exVote is O(tnN(L,l,d)) which is no more than O($tn4^L$), where N(L,l,d) is the number of (l,d)-variants of a length-L sequence and is always less than 4^L. As $k \approx t$ which is normally much larger than L, the time complexity O($tn4^L$) is already much smaller than $O((nt)^{k+2.376})$, the time complexity of the initial step of Styczynski et al.'s algorithm. The improved time complexity rendered by Algorithm exVote is demonstrated in Section 4.

The space complexity for Algorithm exVote is O(4^L + tn) and would not create much problem when L is small. As shown in Figure 1, the value of N(L,l,d) increases exponentially with the motif length L so as the time and space complexities too. In order to handle large L, Section 3.2 describes some simple techniques to reduce the space complexity without increasing the time complexity too much.

3.2. Reducing the space required

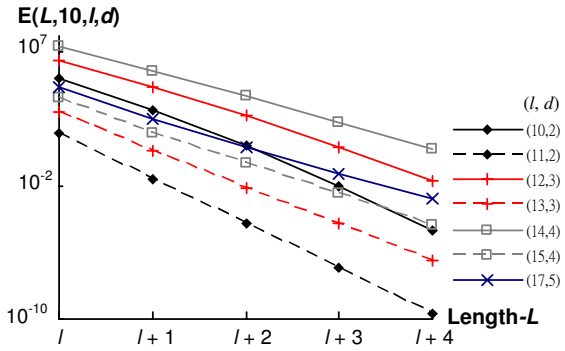
A straight-forward implementation of Algorithm exVote requires $O(4^L)$ space to store the number of votes received by the 4^L length-L sequences. Under normal circumstances where motifs have length $L \le 18$, $4^L \le 4^{18}$ = 64GB memory is still feasible. However, $O(4^L)$ space may be too large for some applications. However, the amount of required space can be reduced by partitioning

Figure 1. Number of (*l*,*d*)-variants of a length-*L* sequence (N(*L*,*l*,*d*)).



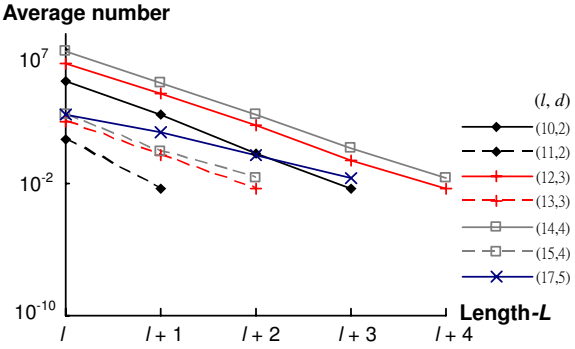
The values of t and n are 20 and 600 respectively.

Figure 2. Expected number of length-*L* motifs with at least 10 (*l*,*d*)-variants E(*L*,*k*,*l*,*d*) by calculation.



The values of t and n are 20 and 600 respectively.

Figure 3. Average number of length-*L* motifs with at least 10 (*l*,*d*)-variants from experiments.



The values of t and n are 20 and 600 respectively.

the 4^L length-L sequences into groups and processing each group one after another. For example, we may partition the sequences into 4^m groups according to their length-m prefix. Two sequences s_1 and s_2 are in the same group if and only if their first m nucleotides are the same.

Instead of scanning the input sequences once, we scan the input sequences 4^m times. For each scan, one length-m prefix P is chosen and all length-L substrings give one vote to each of their (l,d)-variants having prefix P. The space needed to store the votes received by all length-L sequences with prefix P is $O(4^{L-m})$. At the end of each scan, we will find all length-L motifs with prefix P and the space can be reused again for other scans. The time and space complexities of this modified Algorithm exVote will then be $O(tn\mathrm{N}(L,l,d) + tn4^m)$ and $O(4^{L-m} + tn)$ respectively. Note that when $m \leq \log_4 \mathrm{N}(L,l,d)$, the time complexity remains $O(tn\mathrm{N}(L,l,d))$.

3.3. Solving FEMP

In order to solve FEMP, we first find all motifs of different lengths L, each with k (l,d)-variants in the input sequences. We then output only those motifs with $\mathrm{E}(L,k,l,d)$ values less than some predefined threshold α.

In practice, we discover motifs starting from length l to n. Intuitively, when n, t, l, d, and k are fixed, the expected number of length-L motif candidates decreases with L as shown in Figures 2 and 3. When L is large, instead of discovering all length-L motifs directly by voting as described in Section 3.1. We may discover all length-L motifs after knowing the length-(L-1) motifs and checking whether these length-(L-1) motifs can be extended to length-L motifs. This extension step can be done by matching the length-(L-2) prefix of a length-(L-1) motif with the length-(L-2) suffix of another and checking whether their corresponding sets of (l,d)-variants have similar property. Since there are four alphabets in DNA sequences, the expected number of length-L motifs we have to check is $4 \cdot \mathrm{E}(L\text{-}1,k,l,d)$, which is much less than $(\mathrm{E}(L\text{-}1,k,l,d))^2$ in Styczynski et al.'s algorithm.

This extension step will be repeated until we cannot find any motifs for a particular length. In the worst case, we have to discover all motifs of length l to n. However, since $\mathrm{E}(L,k,l,d)$ decreases with L exponentially, we rarely need to go much beyond the length of the planted motif in practice. Therefore, we can discover the planted motif, whose $\mathrm{E}(L,k,l,d)$ value is less than the threshold α, in reasonable time.

4. Experimental Results

We have implemented Algorithm exVote in C++. Experimental results of this algorithm on both simulated data and real biological data will be shown below. Even though our experiments were performed on a P3 700 CPU with 4GB memory, our programs used less than 70MB memory in all experiments.

4.1. Verifying the formula for the expected number of (l,d)-variants

In order to check whether the estimation of E(L,k,l,d) in Section 2.1 is correct, we generated 20 length-600 sequences with equal occurrence probability for each nucleotide and counted the number of length-L sequences having at least k (l,d)-variants. For each set of parameters (l,d), we repeated each experiment 100 times. Figures 2 and 3 show the values of E(L,10,l,d) from the formula given in Section 2.1 and from experiments.

The resemblance of these two figures means that our calculated E(L,10,l,d) is almost the same as the average number of length-L sequences having at least 10 (l,d)-variants. However, for those situations when E(L,10,l,d) is less than 1/100 (e.g E(13,10,11,2) when (l,d)=(11,2) and L=l+2=13), we cannot discover any length-L sequences having at least 10 (l,d)-variants in the 100 experiments. Therefore we cannot plot the values of E(L,10,l,d) in these situations in Figure 3.

We have also performed the same experiments for different values of k. The average numbers of length-L sequences having at least k (l,d)-variants for different k are almost the same as those values of E(L,k,l,d) expressed in our formula in Section 2.1.

4.2. Simulated data

We are interested in comparing the performance of Algorithm exVote against the best performing algorithm up to now. In [22], Styczynski et al. compared the performances of their algorithm with different motif discovering programs like GibbsDNA [6], WINNOWER [17], SP-STAR [17] and PROJECTION [3]. Styczynski et al.'s algorithm has the best performance as it can discover the planted motifs in all cases and has the best accuracy. Thus, in this paper, we only compare Algorithm exVote with Styczynski et al's algorithm. Another popular software PROJECTION, which discovers motifs by heuristic search. The simulated data were generated in the same way as [3], i.e. a total of 20 length-600 sequences (as in Section 4.1), each with a planted variant from a randomly generated M. We repeated the experiments 20 times for each set of parameters (l,d) on Styczynski et al's algorithm, PROJECTION and Algorithm exVote.

Table 1. Accuracies and running times comparison.

(l,d)	PROJECTION		Styczynski et al.'s algorithm		exVote	
	accuracy	(time)	accuracy	(time)	accuracy	(time)
(10,2)	0.82	(161.1s)	1.00	(8 min)	1.00	(0.1 s)
(11,2)	0.95	(12.5 s)	1.00	(< 1 min)	1.00	(0.7 s)
(12,3)	0.71	(8.7 min)	1.00	(10.5 h)	1.00	(9.8 s)
(13,3)	0.94	(46.0 s)	1.00	(10 min)	1.00	(17.4 s)
(14,4)	0.65	(15.4 min)	1.00	(> 3 months)	1.00	(197.5 s)
(15,4)	0.90	(129.0 s)	1.00	(6 h)	1.00	(206.1 s)
(17,5)	0.86	(273.2 s)	1.00	(3 weeks)	1.00	(27 min)

The values of t and n are 20 and 600 respectively

In all cases, both Styczynski et al's algorithm and Algorithm exVote discover the planted motif M.

However, Algorithm exVote is much faster than Styczynski et al's algorithm. Although PROJECTION [3] has the shortest running time for some input parameters, it cannot discover the planted motifs in some cases. Table 1 shows the running time of these three algorithms, in particular, when compared with the running time of Styczynski et al's algorithm, Algorithm exVote has reduced the time needed for solving the (14,4)-problem is reduced from 3 months to 197.5 seconds and from 3 weeks to 27 minutes on the (17,5)-problem.

4.3. Real biological data

The performance of Algorithm exVote was also tested on real biological data. SCPD [25] is a database of different transcription factors for yeast. For each transcription factor, the published motif pattern, the positions of the binding sites and the set of sequences containing these binding sites are kept. We used PROJECTION [3], Voting algorithm [4] (developed for solving the PMP) and Algorithm exVote to discover the motif for each data set. Since both PROJECTION and Voting algorithm needed the motif length l and Hamming distance d as input parameters, we set l be length of the published motif and tested all values of d from 0 to l. These two algorithms are said to have discovered the motif if the published motif can be found for some d. As Algorithm exVote, we adopted l = 6 and d = 1 for all data set.

Table 2 shows the results of these algorithms. Even without specifying the lengths of published motifs, Algorithm exVote can discover all the published motifs. Besides, since the FEMP is more flexible than the PMP,

Table 2. Experimental results on real biological data

Name	Motif pattern	PROJECTION	Voting	Algorithm exVote
AP1	TTANTAA	-	TTACTAA	TTAATAA
BAS1	TGACTC	TGACTC	TGACTC	TGACTC
GATA	CTTATC	CTTATC	CTTATC	CTTATC
GCR1	C[AT]TCC	CTTCC	CTTCC	CTTCC
HSE,HTSF	TTTCTAGAA	TTCTAGAAG	TTTCTAGAA	TTTCTAGAA
NBF	ATG[CT]G[AG]A [AT][AT]	TGTGAAAAG	ATGTGAAAA	ATGTGAAAT
ROX1	[CT][CT]NATTGTT[CT]	-	-	ATTGTTC
UASGABA	AAAAACCGCCGGCGGCAAT	-	-	AAAAGCCGCCGGCGGCAAT

in some cases (ROX1 and UASGABA), Algorithm exVote can discover the correct motifs while PROJECTION and Voting algorithm fail. The performances of these three algorithms for the other transcription factors in SCPD have been omitted because they have similar performances for these data, i.e. all of them fail to find the motifs or all of them can find the same motifs).

5. Discussion

In this paper, we introduced the expected number of sequences with similar properties, in a set of random sequences of the same number and length, as a measure for comparing different motifs. Based on this measure, we define the Further Extended (*l*,*d*)-Motif Problem (FEMP) which does not need to take the motif's length and the number of planted variants as input parameters.

We have also developed Algorithm exVote to solve the problems EMP and FEMP. Experiments on simulated data and real biological data show that Algorithm exVote performs better than the popular motif discovering algorithms in terms of flexibility and running time.

The main purpose of the "maximal" motif is to reduce the number of motifs to be considered, by eliminating similar or inferior ones. However, discovering maximal motifs may not be the best way for solving some of the motif problems. For example, given the following sequences

```
    0123456789
S1: TATTCACTGC...
S2: GCCTCACCTG...
S3: CGGACATGAC...
```

We want to solve the Extended (4,1)-Motif Problem with $k = 3$. "GTCAT" has 3 (4,1)-variants at $S_i[2\ldots6]$ and "ACACG" at $S_i[3\ldots7]$ for $i = 1, 2, 3$. "ACACG" is not a maximal motif because it is of the same length and has the same number of variants as "GTCAT" and the positions of its variants are one base-pair behind "GTCAT" (Property 4). However, they are treated as the same even though "GTCAT" and "ACACG" are two motifs with very different patterns. Thus, it is doubtful whether Property 4 should be included in the definition of maximal motif. Note that Algorithm exVote can discover all maximal motifs with the same time and space complexities without Property 4.

In the future, we will study how to determine the values of *l* and *d* effectively such that users do not need to determine the values of any parameters.

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Appendix: Calculating N(*L*,*l*,*d*)

In this section, we will describe how to calculate the number N(*L*,*l*,*d*) of (*l*,*d*)-variants of a length-*L* sequence *M*. Given two length-*L* sequences σ and *M*, $e_{suf}(\sigma, M)$ is a length-*l* bit string defined as follows,

$$e_{suf}(\sigma,M)[i]=\begin{cases}1 & \sigma[L-l+i]\neq M[L-l+i]\\ 0 & \sigma[L-l+i]=M[L-l+i]\end{cases}$$

Let C[*L*, β] be the number of (*l*,*d*)-variants σ of a length-*L* sequence *M* satisfying $e_{suf}(\sigma, M) = \beta$. For example, when *l* = 5, given two length-8 sequences

```
          12345678
σ:        AGCTAACG
M:        CCGTTACT
```

$e_{suf}(\sigma, M)$ = "01001" which represents the positions that the suffix of σ and *M* are different.

By considering all possible bit strings β, we can calculate the number of (*l*,*d*)-variants of a length-*L* sequence

$$N(L,l,d)=\sum_{\beta}C[L,\beta]=\sum_{\beta:|\beta|\le d}C[L,\beta]$$

We applied dynamic programming to calculate C[*L*, β]. When *L* = *l*, we have

$$C[l,\beta]=\begin{cases}3^{|\beta|} & |\beta|\le d\\ 0 & |\beta|>d\end{cases}$$

where $|\beta|$ be the number of '1' in the bit string β. When *L* > *l*, we calculate the value of C[*L*, β] by the following recurrence,

$$C[L,\beta]=\begin{cases}3\left(C[L\text{-}1,0\bullet\beta']+C[L\text{-}1,1\bullet\beta']\right) & \beta[l]=1\wedge|\beta'|<d\\ C[L\text{-}1,0\bullet\beta']+C[L\text{-}1,1\bullet\beta'] & \beta[l]=0\wedge|\beta'|\le d\\ 0 & |\beta|>d\end{cases}$$

where $\beta'=\beta[1...l-1]$ and $\bullet$ means concatenate

Although there are 2^l possible length-*l* bit strings β, we only consider the values of those C[*L*, β] with $|\beta| \le d$. Therefore the total number of C[*L*, β] we have to calculate is at most

$$(L-l+1)\sum_{j=0}^{d}\binom{l}{j}$$

Since it takes constant time to calculate each C[*L*, β], the total running time for counting N(*L*,*l*,*d*) is

$$O\left((L-l+1)\sum_{j=0}^{d}\binom{l}{j}+\sum_{j=0}^{d}\binom{l}{j}\right)=O\left((L-l)\sum_{j=0}^{d}\binom{l}{j}\right)$$ □