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A SEQUENTIAL METHOD FOR DISCOVERING PROBABILISTIC MOTIFS IN PROTEINS

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Abstract: This paper proposes a greedy algorithm for learning a mixture of motifs model through likelihood maximization, in order to discover common sub-strings, known as motifs, to a given collection of related biosequences. The approach adds sequentially a new motif to a mixture model by performing a combined scheme of global and local search for appropriately initializing its parameters. In comparison with the well-known MEME approach, the algorithm is advantageous in terms of identifying motifs with significant conservation and the development of larger protein fingerprints.

Keywords: motif discovery, mixture of motifs, EM algorithm, fingerprints, MEME algorithm.

I. INTRODUCTION

The motif identification is one of the most important problems in protein sequence analysis covering many application areas. It concerns the discovery of portions of protein strands of major biological interest with important structural and functional features. Motifs can also be used for characterizing biological families and searching for new family members. This leads to the development of diagnostic signatures (fingerprints) that contain groups of conserved motifs used to characterize a family. The PRINTS (or PRINT-S) database [1] is an example of protein fingerprints database containing ungapped motifs. Usually, patterns or motifs can be either deterministic or probabilistic [6]. A simplified way of modeling a probabilistic ungapped motif is the position weight matrix (PWM) representing the relative frequency of each character at each motif position. The Gibbs sampling [4] and MEME [2] represent probabilistic methods for finding multiple shared motifs within a set of unaligned biological sequences. The MEME algorithm fits a two-component finite mixture model to a set of sequences using the Expectation Maximization (EM) algorithm [5], where one component describes the motif and the other describes the background (other positions in the sequences). Multiple motifs are discovered by sequentially applying a new mixture model with two components to the sequences remaining after erasing the occurrences of the already identified motifs.

In this paper we present an innovative approach for discovering significant motifs in a set of sequences based on recently developed incremental schemes for Gaussian mixture learning [7]. Our method learns a mixture of motifs model in a greedy fashion by incrementally adding components (motifs) to the mixture. Starting with one component that models the background, at each step a new component is added which corresponds to a candidate motif. The algorithm tries to identify a good initialization for the parameters of the new motif by performing global search over the input substrings together with local search for fine tuning of the parameters of the new component. In addition, a hierarchical clustering procedure is proposed based on kd-tree techniques [3] for partitioning the input dataset of substrings, which can reduce the time complexity for global searching.

II. GREEDY EM ALGORITHM FOR MOTIFS DISCOVERY

A. The mixture of motifs model

Consider a finite set of characters $\Sigma = \{\alpha_1, \ldots, \alpha_L\}$ where $\Omega = [\Sigma]$. Any sequence $S = a_1a_2 \ldots a_L$ of length $L$, such that $L \geq 1$ and $a_i \in \Sigma$, is called a string (or sequence) over the character set $\Sigma$. The consecutive characters $a_i \ldots a_{i+W-1}$ form a substring $x_i$ of length $W$, identified by the starting position $i$ over the string $S$. There are $n = L - W + 1$ such possible substrings of length $W$ generated from sequence $S$. We assume a set of $N$ unaligned sequences $S = \{S_1, \ldots, S_N\}$ of length $L_1, \ldots, L_N$, respectively. In order to deal with the problem of motif discovery of length $W$ we construct a new dataset containing all substrings of length $W$ in $S$. Therefore, we obtain a training dataset $X = \{x_1, \ldots, x_n\}$ of $n$ substrings ($n = \sum_{i=1}^{N} (L_i - W + 1)$) for the learning problem.

A mixture of motifs model $f$ for an arbitrary substring $x_i$ assuming $g$ components can be written as:

$$f(x_i; \Psi_g) = \sum_{j=1}^{g} \pi_j \phi_j(x_i; \theta_j),$$

(1)

where $\Psi_g$ is the vector of all unknown parameters in the mixture model of $g$ components, i.e. $\Psi_g = [\pi_1, \ldots, \pi_{g-1}, \theta_1, \ldots, \theta_g]$. The mixing proportion $\pi_j$ ($\pi_j \geq 0$) can be viewed as the prior probability that data
$x_i$ has been generated by the $j$th component of the mixture and they satisfy $\sum_{j=1}^{g} \pi_j = 1$.

Each one of the $g$ components corresponds to either a motif or the background. Following the position weight matrix representation, a motif $j$ can be modeled by $PWM_j = [p_{j,k}^l]$ of size $(\Omega \times W)$, where each value $p_{j,k}^l$ denotes the probability that the letter $\alpha_l$ is located in motif position $k$. On the other hand, a background component $j$ is represented using a probability vector $BPM_j$ (of length $\Omega$), where each parameter value $\theta_j^l$ denotes the probability of letter $\alpha_l$ to occur at an arbitrary position. The probability that a substring $x_i = \alpha_{i1} \ldots \alpha_{iW}$ has been generated by the component $j$ is

$$\phi_j(x_i; \theta_j) = \begin{cases} \prod_{l=1}^{W} p_{j,k}^l & \text{if } j \text{ is motif} \\ \prod_{l=1}^{W} \theta_j^l & \text{if } j \text{ is background} \end{cases}$$ (2)

The log-likelihood of the observed dataset $X$ corresponding to the above model is

$$L(\Psi_g) = \sum_{i=1}^{n} \log f(x_i; \Psi_g).$$ (3)

Formulating the problem as an incoherent-data problem [5], each substring $x_i$ can be considered as having arisen from one of the $g$ components of the mixture model of Equation 1. Therefore, we can introduce the parameters $z_{ij} = 1$ or $0$ that indicate whether $x_i$ has been generated by the $j$-th component of the mixture. The EM algorithm can be applied for the log-likelihood maximization problem by treating the $z_{ij}$ as missing data. The following update equations are obtained for each component $j$ [2, 5]

$$z_{ij}^{(t+1)} = P_r(x_{ij} = 1|x_i, \Psi_g^{(t)}) = \frac{\pi_j^{(t)} \phi_j(x_i; \theta_j^{(t)})}{\sum_{j=1}^{g} \pi_j^{(t)} \phi_j(x_i; \theta_j^{(t)})}$$ (4)

$$z_{ij}^{(t+1)} = \frac{1}{n} \sum_{i=1}^{n} z_{ij}^{(t+1)},$$ (5)

$$\theta_j^{(t+1)} = \begin{cases} \frac{\sum_{i=1}^{n} z_{ij}^{(t+1)} \theta_j^l}{\sum_{i=1}^{n} z_{ij}^{(t+1)}} & \text{if } j \text{ is motif} \\ \frac{\sum_{i=1}^{n} z_{ij}^{(t+1)} \theta_j^l}{\sum_{i=1}^{n} z_{ij}^{(t+1)}} & \text{if } j \text{ is background} \end{cases}$$ (6)

where the elements $\theta_j^l$ ($\theta_j^l$) correspond to the observed frequency of letter $\alpha_l$ at position $k$ of motif $j$ occurrences (at background $j$ arbitrary positions) and can be formally expressed as

$$\theta_j^l = \sum_{k=1}^{W} z_{ij}^{(t+1)} \sum_{l=1}^{1} I(\alpha_{lk}, l)$$

The indicator $I(\alpha_{lk}, l)$ denotes a binary function which takes value 1 if the substring $x_i$ contains letter $\alpha_l$ at position $k$ $(\alpha_{lk} \equiv \alpha_l)$ and 0 otherwise.

Equations 4-6 can be used to estimate the parameter values $\Psi_g$ of the $g$-component mixture model which maximize the log-likelihood function and ensure the convergence of the algorithm to a local maximum of the likelihood function [5]. However, its great dependence on parameter initialization and its local nature (it gets stuck in local maxima of the likelihood function) do not allow us to directly apply the EM algorithm to a $g$ component mixture of motifs model. To overcome the problem of poor initialization, we next propose an efficient alternative scheme of global searching over appropriate defined candidate motifs, followed by a local searching for fine tuning the parameters of a new motif.

### B. Greedy mixture learning

Assume that a new component $\phi_{g+1}(x_i; \theta_{g+1})$ that corresponds to a motif is added to a $g$-component mixture model $f(x_i; \Psi_g)$. Then the resulting mixture has the following form

$$f(x_i; \Psi_{g+1}) = (1-\alpha)f(x_i; \Psi_g) + \alpha \phi_{g+1}(x_i; \theta_{g+1}),$$ (7)

where $\alpha \in (0, 1)$. The vector $\Psi_{g+1}$ specifies the new parameter vector and consists of the parameter vector $\Psi_g$ of the $g$-component mixture, the weight $\alpha$ and the parameter vector $\theta_{g+1}$. This formulation proposes a two-component likelihood maximization problem, where the first component is described by the old mixture $f(x_i; \Psi_g)$ and the second one is the motif component $\phi_{g+1}(x_i; \theta_{g+1})$. If we consider that the parameters $\Psi_g$ of $f(x_i; \Psi_g)$ remain fixed during maximization of $L(\Psi_{g+1})$, the problem can be treated by applying searching techniques to optimally specify the parameters $\alpha$ and $\theta_{g+1}$ which maximize $L(\Psi_{g+1})$.

As presented in [7], an EM algorithm can be applied where the learning procedure is applied only to the mixing weight $\alpha$ and the probabilistic quantities $\theta_{g+1}$ of the newly inserted component. Following Equations 5-7, the next update procedures can be derived

$$z_{i1,g+1} = \frac{\alpha \phi_{g+1}(x_i; \theta_{g+1})}{(1-\alpha)f(x_i; \Psi_g) + \alpha \phi_{g+1}(x_i; \theta_{g+1})}$$

$$z_{i1,g+1} = \frac{1}{n} \sum_{i=1}^{n} z_{i1,g+1},$$ (9)

$$\theta_{g+1}^{(t+1)} = [\theta_{g+1}^{(t+1)}],$$ (10)

where

The above partial EM steps constitute a simple and fast method for local searching the maxima of $L(\Psi_{g+1})$. However, the problem of poor initialization still remains since this scheme is very sensitive to the proper initialization of the two parameters $\alpha$ and $\theta_{g+1}$. For this reason
global search strategy has been developed [7] which substitutes the log-likelihood function using a Taylor approximation about a point \( a = a_0 \), and then using the resulting estimate to search for the optimal \( \theta_{g+1} \) value. Therefore we expand \( \mathcal{L}(\Psi_{g+1}) \) by second order Taylor expansion about \( a_0 = 0.5 \) and then the resulting quadratic function is maximized with respect to \( a \). It can be shown [7] that, for a given parameter vector \( \theta_r \), a local maximum of \( \mathcal{L}(\Psi_{g+1}) \) near \( a_0 = 0.5 \) is given by

\[
\hat{\theta}_r = \frac{1}{2} \frac{\sum_{i=1}^{n} \delta(x_i; \theta_r)}{\sum_{i=1}^{n} \delta^2(x_i; \theta_r)},
\]

and is obtained for

\[
\hat{\theta}_r = \frac{1}{2} \frac{\sum_{i=1}^{n} \delta(x_i; \theta_r)}{\sum_{i=1}^{n} \delta^2(x_i; \theta_r)},
\]

where

\[
\delta(x_i; \theta_r) = \frac{f(x_i; \Psi_g) - \phi_{g+1}(x_i; \theta_r)}{f(x_i; \Psi_g) + \phi_{g+1}(x_i; \theta_r)},
\]

The above methodology has the benefit of modifying the problem of maximizing the likelihood function to become independent on the selection of initial value for the mixing weight \( a \). The only problem now is the identification of candidate values \( \theta_r \) so as to properly initialize the motif parameters and to conduct partial EM steps.

A reasonable approach is to search for candidates directly over the total dataset of substrings \( X = \{ x_r \} \), \( (r = 1, \ldots, n) \). For this reason we associate with each substring \( x_r = x_1 \ldots x_W \) a position weight matrix \( \theta_r \), constructed as follows

\[
\theta_r = [p_{r,k}], \quad \text{where} \quad p_{r,k} = \begin{cases} \lambda & \text{if } x_{i-k} = x_i \\ \frac{1-\lambda}{W-1} & \text{otherwise} \end{cases},
\]

where the parameter \( \lambda \) has a fixed value in the range \((0, 1)\). Therefore, the log-likelihood \( \mathcal{L}(\theta_r) \) is determined by selecting among the \( \theta_r \) matrices the one which maximizes the right hand size of Equation 11.

The drawback of the above approach is the increasing time complexity \( (O(n^2)) \) of the search procedure. In order to reduce the complexity, we perform a hierarchical clustering technique based on the notion of \( kd \)-trees [3], by proposing a modified approach in order to deal with sequential symbolical data. In particular, using an appropriate criterion based on maximum character variance, we apply a partitioning scheme that divides the original set \( X \) into a set of \( C \ll n \) clusters. The position weight matrices (Equation 14) corresponding to the centroids of the clusters (consensus substrings) constitute the candidate matrices \( \theta_r \) \( (r = 1, \ldots, C) \) used in global search (Equations 11 - 13).

### Table 1: The seed substrings in the artificial datasets

<table>
<thead>
<tr>
<th>m</th>
<th>seed motifs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A VER Y INTERESTING VIEW</td>
</tr>
<tr>
<td>2</td>
<td>D EST INATED DEDICATED</td>
</tr>
<tr>
<td>3</td>
<td>P R IMER STAGE DESIGNING</td>
</tr>
<tr>
<td>4</td>
<td>W AITING FOR THIS GIANT TRAIN</td>
</tr>
<tr>
<td>5</td>
<td>S T W A R T E N E M P L E N A R M</td>
</tr>
<tr>
<td>6</td>
<td>A R I S T I D IS K H A N I L E H A M</td>
</tr>
</tbody>
</table>

Special treatment has also been given to avoid overlapping with the already discovered motifs during the selection of a candidate motif instance. This is achieved by excluding, from the set \( C \) of consensus substrings (candidate motifs), those substrings that overlap with motif occurrences.

### III. EXPERIMENTAL RESULTS

In order to evaluate the effectiveness of our method we have conducted a series of experiments considering both artificial and real sets of biological sequences. In all cases the width \( W \) of the motifs is considered constant, while good values for the \( \lambda \) parameter used to initialize the candidate position weight matrices (Equation 14), were found to be in the range \([0.6, 0.8]\). For all the experimental datasets we have also applied the MEME approach using the available software from the corresponding Web site.

**Experiments with artificial datasets**

In the artificial datasets used in our experiments each motif has an associated randomly generated "seed substring" and copies of the motif are created by randomly performing a number of substitutions (mutations) with a mutation probability \( p_m \). We created artificial sequences of variable length (between 310 and 330) by randomly locating (ensuring no overlapping) and mutating copies of six \( (6) \) different seed substrings of length \( W = 20 \) (Table 1). The rest positions were filled with characters from the amino acids (AA) alphabet \((\Omega = 20)\). As illustrated in table 1 the last two seed substrings (5 and 6) are exactly the same in half of their length (from position 11 to 20). Assuming three different values of the mutation probability \( \{0, 0.1, 0.2\} \), three different datasets of twenty \((N = 20)\) artificial protein sequences were constructed. The comparative results with the MEME algorithm have shown the superiority of the greedy EM algorithm in discovering all the incorporated motifs in the three datasets. The MEME approach was unable to identify the motifs 5 and 6 and considered them as one motif.

**Experiments with real datasets**

The real datasets used in our experiments were obtained from the PRINTS database \([1]\) which contains protein

\[1\] The Web site of MEME/MAST system version 3.0 can be found at http://meme.sdsc.edu/meme/website/
<table>
<thead>
<tr>
<th>Problem</th>
<th>Acc. number</th>
<th>Greedy EM</th>
<th>MEME</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Motif</td>
<td>IC</td>
</tr>
<tr>
<td>PR00058</td>
<td>(W = 20)</td>
<td>1</td>
<td>72.4434</td>
</tr>
<tr>
<td></td>
<td>16 seqs of 297 AA 6 motifs</td>
<td>II</td>
<td>72.4559</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>72.3134</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>67.5361</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>69.4094</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VI</td>
<td>69.7350</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VII</td>
<td>65.3427</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIII</td>
<td>57.0378</td>
</tr>
<tr>
<td>PR00810</td>
<td>(W = 10)</td>
<td>1</td>
<td>37.2183</td>
</tr>
<tr>
<td></td>
<td>6 seqs of 256 AA 2 motifs</td>
<td>II</td>
<td>35.2812</td>
</tr>
<tr>
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<td>III</td>
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<td></td>
<td>V</td>
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<td></td>
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<td>VI</td>
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<tr>
<td>PR01268</td>
<td>(W = 17)</td>
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<tr>
<td></td>
<td>19 seqs of 209 AA 3 motifs</td>
<td>II</td>
<td>57.0125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
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<td>V</td>
<td>56.2635</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VI</td>
<td>54.2635</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VII</td>
<td>51.4655</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIII</td>
<td>50.0629</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IX</td>
<td>53.1297</td>
</tr>
</tbody>
</table>

motif fingerprints. Three families from the PRINTS database were selected, describing fingerprints for L5 ribosomal proteins (PR00058), secretion pathway protein C (PR00810) and pi-class glutathione S-transferases (PR001268). Each motif discovered was evaluated in terms of the information content (IC) [2], specified as follows

$$IC_j = \sum_{k=1}^{W} \sum_{\alpha_i \in B} p_i^j \log_2 \frac{p_i^j}{p_i^k}$$  

(15)

where $p_i^j$ indicates the overall background probability of letter $\alpha_i$ in the dataset. This score becomes maximal if the motif is well conserved.

Table 2 summarizes the comparative results obtained using the three protein families. The superiority of the greedy EM algorithm over MEME is obvious not only in terms of the greater number of real-motifs discovered but also in terms of the degree of motif conservations as indicated by the IC scores. In all cases, the number of the discovered motifs is also greater than the number of motifs specified in the PRINTS database (Table 2). This means that the proposed method has led to the discovery of larger fingerprints (containing more motifs) and thus constitutes a promising tool for biological sequence analysis.

IV. CONCLUSIONS

In this paper we have proposed a greedy EM algorithm for solving the multiple motif discovery problem in biological sequences. Our approach learns a mixture of motifs model in a greedy fashion by iteratively adding new components, through a combined scheme of local and global search which ensures fine tuning of the parameter vector of the new component.

The main difference with the MEME technique is the way that the mixture models are applied. Although both methods treat the same problem through mixture learning using the EM algorithm, our approach is able to effectively fit multiple-component mixture models, overcoming the problem of poor initialization of EM that frequently gets stuck on local maxima of the likelihood function. This results in exploring the input dataset efficiently and the discovery of greater number of motifs. The MEME scheme of erasing motif occurrences, pruning in such way the input dataset, does not allow the parameters of the discovered motifs to be reestimated, and thus future discovered motifs cannot contribute to possible re-allocation of the character distribution in the motif positions. As the results indicate, this drawback becomes significant in cases where they exist motifs that partially match, since these motifs are recognized by the MEME algorithm as one "composite" motif that cannot be further analyzed.

REFERENCES


