Biological Sequence Analysis

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Abstract

This talk will review a little over a decade's research on applying certain stochastic models to biological sequence analysis. The models themselves have a longer history, going back over 30 years, although many novel variants have arisen since that time. The function of the models in biological sequence analysis is to summarize the information concerning what is known as a motif or a domain in bioinformatics, and to provide a tool for discovering instances of that motif or domain in a separate sequence segment. We will introduce the motif models in stages, beginning from very simple, non-stochastic versions, progressively becoming more complex, until we reach modern profile HMMs for motifs. A second example will come from gene finding using sequence data from one or two species, where generalized HMMs or generalized pair HMMs have proved to be very effective.

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1. Introduction

DNA (deoxyribonucleic acid), RNA (ribonucleic acid), and proteins are macromolecules which are unbranched polymers built up from smaller units. In the case of DNA these units are the 4 nucleotide residues A (adenine), C (cytosine), G (guanine) and T (thymine) while for RNA the units are the 4 nucleotide residues A, C, G and U (uracil). For proteins the units are the 20 amino acid residues A (alanine), C (cysteine) D (aspartic acid), E (glutamic acid), F (phenylalanine), G (glycine), H (histidine), I (isoleucine), K (lysine), L (leucine), M (methionine), N (asparagine), P (proline), Q (glutamine), R (arginine), S (serine), T (threonine), V (valine), W (tryptophan) and Y (tyrosine). To a considerable extent, the chemical properties of DNA, RNA and protein molecules are encoded in the linear sequence of these basic units: their primary structure.

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The use of statistics to study linear sequences of biomolecular units can be descriptive or it can be predictive. A very wide range of statistical techniques has been used in this context, and while statistical models can be extremely useful, the underlying stochastic mechanisms should never be taken literally. A model or method can break down at any time without notice. Further, biological confirmation of predictions is almost always necessary.

The statistics of biological sequences can be global or it can be local. For example, we might consider the global base composition of genomes: \( E. \text{ coli} \) has 25% A, 25% C, 25% G, 25% T, while \( P. \text{ falciparum} \) has 82%A+T. At the very local, the triple ATG is the near universal motif indicating the start of translation in DNA coding sequence. A major role of statistics in this context is to characterize individual sequences or classes of biological sequences using probability models, and to make use of these models to identify them against a background of other sequences. Needless to say, the models and the tools vary greatly in complexity.

Extensive use is made in biological sequence analysis of the notions of motif or domain in proteins, and site in DNA. We shall use these terms interchangeably to describe the recurring elements of interest to us. It is important to note that while we focus on the sequence characteristics of motifs, domains or sites, in practice they also embody (biochemical) structural significance.

2. Deterministic models

The C2H2 (cysteine-cysteine histidine-histidine) zinc-finger DNA binding domain is composed of 25-30 amino acid residues including two conserved cysteines and two conserved histidines spaced in a particular way, with some restrictions on the residues in between and nearby. Of course the arrangement reflects the three-dimensional molecular structure into which the amino-acid sequence folds, for it is the structure which has the real biochemical significance, see Figure 1, which was obtained from [http://www.rcsb.org/pdb/](http://www.rcsb.org/pdb/) An example of this motif is the 27-
letter sequence known as 1ZNF, this being a Protein Data Bank identifier for the structure XFIN-31 of *X. laevis*. Its amino acid sequence is

1ZNF: XYKCGLCERSFVEKSALSRHQRVHKNX

Note the presence of the two Cs separated by 2 other residues, and the two Hs separated by 3 other residues. Here and elsewhere, X denotes an arbitrary amino acid residue. A popular and useful summary description of C2H2 zinc fingers which clearly includes our example, is the regular expression

\[
C \cdash X(2, 4) \cdash C \cdash X(3) \cdash [LIVMFYW\text{C}] \cdash X(8) \cdash H \cdash X(3, 5) \cdash H
\]

where \(X(m)\) denotes a sequence of \(n\) unspecified amino acids, while \(X(m, n)\) denotes from \(m\) to \(n\) such, and the brackets enclose mutually exclusive alternatives. There is a richer set of notation for regular expressions of this kind, but for our purposes it is enough to note that this representation is essentially deterministic, with uncertainty included only through mutually exclusive possibilities (e.g. length or residue) which are not otherwise distinguished.

Simple and efficient algorithms exist for searching query sequences of residues to find every instance of the regular expression above. In so doing with sequence in which all instances of the motif are known, we may identify some sub-sequences of the query sequence which are not C2H2 zinc finger DNA binding domains, i.e. which are false positives, and we may miss some sub-sequences which are C2H2 zinc fingers, i.e. which are false negatives. Thus we have essentially deterministic descriptions and search algorithms for the C2H2 motifs using regular expressions. Their performance can be described by the frequency of false positives and false negatives, equivalently, their complements, the specificity and sensitivity of the regular expression. We do not have space for an extensive bibliography, so for more on regular expressions and on most of the other concepts we introduce below, see [2].

3. Regular expressions can be limiting

Most protein binding sites are characterized by some degree of sequence specificity, but seeking a consensus DNA sequence is often an inadequate way to recognize their motifs. Simply listing the alternatives seen at a position may not be very informative, but keeping track of the frequencies with which the different alternatives appear can be very valuable. Thus position-specific nucleotide or amino acid distributions came to represent the variability in DNA or protein motif composition. This is just the set of marginal distribution of letters at each position. Rather than present an extensive tabulation of frequencies for our C2H2 zinc finger example, we present a pictorial representation: a sequence logo coming from [http://blocks.fhcrc.org](http://blocks.fhcrc.org).

Sequence logos are scaled representation of position-specific nucleotide or amino acid distributions. The overall height at a given position is proportional to information content, which is a constant minus the entropy of the distribution at that
Figure 2: Sequence logo for C2H2 zinc finger

position. The proportions of each nucleotide or amino acid at a position are in relation to their observed frequency at that position, with the most frequent on top, the next most frequent below, etc.

4. Profiles

It is convenient for our present purposes to define a profile as a set of position-specific distributions describing a motif. (Traditionally the term has been used for the derived scores.) How would we use a set of such distributions to search a query sequence for instances of the motif? The answer from bioinformatics is that we score the query sequence, and for suitably large scores, declare that a candidate subsequence is an instance of our motif.

There are a number of approaches for deriving profile scores, but the easiest to explain here is this: scores are log-likelihood ratio test statistics, for discriminating between a probability model $M$ for the motif and a model $B$ for the background. The model $M$ will be the direct product of the position-specific distributions, (i.e. the independent but not identical distribution model), while the background model $B$ will be the direct product of a set of relevant background frequencies (i.e. the independent and identical distribution model). Thus, if $f_{al}$ is the frequency of residue $a$ at position $l$ of the motif, and $f_a$ background frequency of the same residue, then the profile score assigned to residue $a$ at position $l$ in a possible instance of the motif will be $s_{al} = \log \frac{f_{al}}{f_a}$. These scores are then summed across the positions in the motif, and compared to a suitably defined threshold. Note that proper setting of the threshold requires a set of data in which all instances of the motif are known. The false positive and false negative rate could then be
determined for various thresholds, and a suitable choice made.

We briefly discuss variants of the log-likelihood ratio scores. In many contexts, it will matter little whether a position is occupied by a leucine (L) rather than an isoleucine (I), as each can evolve in time to or from the other rather more readily than from other residues. Thus it might make sense to modify the scores to take this and similar evolutionary patterns into account. Indeed the first use of profiles involved scores of this kind, using the position specific amino acid distribution of an alignment of instances of the motif and entries from what are known as PAM matrices, which embody patterns of molecular evolution. In addition, the background distribution of residues may be modelled more detailed manner, e.g. using the so-called Dirichlet mixture models.

It is also possible to include position-specific scores for insertion and deletion of residues, relative to a consensus pattern. When these are used, the scoring becomes a little more subtle, as the problem is then quite analogous to pairwise sequence alignment, but with position dependent scoring parameters for matches, mismatches, insertions and deletions.

We summarise this section by noting that probability has entered into our description through the use of frequencies, and scores based on them, but so far we do not have global statistical models, at least not ones embodying insertions and deletions, on which we base our estimation and testing. These are all part of the use of profile HMMs, but first we introduce HMMs.

5. Hidden Markov models

Hidden Markov models (HMMs) are processes \((S_t, O_t), t = 1, \ldots, T\), where \(S_t\) is the hidden state and \(O_t\) the observation at time \(t\). Their probabilistic evolution is constrained by the equations

\[
\begin{align*}
pr(S_t | S_{t-1}, O_{t-1}, S_{t-2}, O_{t-2}, \ldots) &= pr(S_t | S_{t-1}), \\
pr(O_t | S_{t-1}, O_{t-1}, S_{t-2}, O_{t-2}, \ldots) &= pr(O_t | S_{t}, S_{t-1}).
\end{align*}
\]

The definitions and basic facts concerning HMMs were laid out in a series of beautiful papers by L. E. Baum and colleagues around 1970, see [2] for references. Much of their formulation has been used almost unchanged to this day. Many variants are now used. For example, the distribution of \(O\) may not depend on previous \(S\), or it may also depend on previous \(O\) values,

\[
\begin{align*}
pr(O_t | S_t, S_{t-1}, O_{t-1}, \ldots) &= pr(O_t | S_t), \quad \text{or} \\
pr(O_t | S_t, S_{t-1}, O_{t-1}, \ldots) &= pr(O_t | S_t, S_{t-1}, O_{t-1}).
\end{align*}
\]

Most importantly for us below, the times of \(S\) and \(O\) may be decoupled, permitting the observation corresponding to state time \(t\) to be a string whose length and composition depends on \(S_t\) (and possibly \(S_{t-1}\) and part or all of the previous observations). This is called a hidden semi-Markov or generalized hidden Markov model.
Early applications of HMMs were to finance, but these were never published, to speech recognition, and to modelling ion channels. In the mid-late 1980s HMMs entered genetics and molecular biology, where they are now firmly entrenched. One of the major reasons for the success of HMMs as stochastic models is the fact that although they are substantial generalizations of Markov chains, there are elegant dynamic programming algorithms which permit full likelihood calculations in many cases of interest. Specifically, there are algorithms which permit the efficient calculation of a) \( \text{pr}(\text{sequence}|M) \), where \( \text{sequence} \) is a sequence of observations and \( M \) is an HMM; b) the maximum over states of \( \text{pr}(\text{states}|\text{sequence}, M) \), where \( \text{states} \) is the unobserved state sequence underlying the observation \( \text{sequence} \); and c) the maximum likelihood estimates of parameters in \( M \) based on the observation \( \text{sequence} \). Step c) is carried out by an iterative procedure which in the case of independent states was later termed the EM algorithm.

6. Profile HMMs

In a landmark paper A. Krogh, D. Haussler and co-workers introduced profile HMMs into bioinformatics. An illustrative form of their profile HMM architecture is given in Figure 3. There we depict the underlying state space of the hidden Markov chain of a profile HMM of length 4, with \( M \) denoting match states, \( I \) insert states and \( D \) delete states, while \( B \) and \( E \) are begin and end states, respectively. Encircled states (\( D \), \( B \) and \( E \)) do not emit observations, while each of the match and insert states will have position-specific observation or emission distributions. Finally, each arrow will have associated transition probabilities, with the expectation being that the horizontal transition probabilities are typically near unity. This the chain proceeds from left to right, and if it remains within match states, its output will be an amino acid sequence of length 4. Deviation to the insert or delete states will modify the output accordingly. The similarity with a direct product of a sequence of position-specific distributions should be unmistakeable. The profile HMMs in use now have considerably more features, while sharing the basic \( M, I \) and \( D \) architecture.

![Figure 3: State space of a simple profile HMM](image-url)
Why was the introduction of the HMM formalism such an advance? The answer is simple: it permitted the construction and application of profiles to be conducted entirely within a formal statistical framework, and that really helped. Instances of the motif embodied in an HMM could be identified by calculating \( \text{pr}(\text{sequence}|M)/\text{pr}(\text{sequence}|B) \) as was done with profiles, using the algorithm for problem a) in X above. Instances of the motif could be aligned to the HMM by calculating the most probable state sequence giving rise to the motif sequence, in essence finding the most probable sequence of matches, insertions, deletions which align the given sequence to the others which gave rise to the HMM, cf. problem b) above. And finally, the parameters in the HMMs could be estimated from data comprising known instances of the motif by using maximum likelihood, an important step for many reasons, one being that it put insert and delete scores on precisely the same footing as match and mismatch scores. Although the estimation of HMM parameters is easiest if the example sequences are properly aligned, the EM algorithm (problem c) above) does not require aligned sequences.

In the years since the introduction of profile HMMs, they have become the standard approach to representing motifs and protein domains. The database Pfam (http://pfam.wustl.edu) now has 3,849 hidden Markov models (May 2002) representing recognized protein or DNA domains or motifs. Profile HMMs have essentially replaced the use of regular expressions and the original profiles for searching other databases to find novel instances of a motif, for finding a motif or domain match to an input sequence, and for aligning a motif or domain to a an existing family. There is considerable evidence that the HMM-based searches are more powerful than the older profile based ones, though they are slower computationally, and at times that is an important consideration.

7. Finding genes in DNA sequence

Identifying genes in DNA sequence is one of the most challenging, interesting and important problems in bioinformatics today. With so many genomes being sequenced so rapidly, and the experimental verification of genes lagging far behind, it is necessary to rely on computationally derived genes in order to make immediate use of the sequence.

What is a gene? Most readers will have heard of the famous central dogma of molecular biology, in which the hereditary material of an organism resides in its genome, usually DNA, and where genes are expressed in a two-stage process: first DNA is transcribed into a messenger RNA (mRNA) sequence, and later a processed form of this sequence is translated into an amino acid sequence, i.e. a protein. In general the transcribed sequence is longer than the translated portion: parts called introns (intervening sequence) are removed, leaving exons (expressed sequence), of which only some are expressed, while the rest remain untranslated. The translated sequence comes in triples called codons, beginning and ending with a unique start (ATG) and one of three stop (TAA, TAG, TGA) codons. There are also characteristic intron-exon boundaries called splice donor and acceptor sites, and a variety of other motifs: promoters, transcription start sites, polyA sites, branching sites, and
so on.

All of the foregoing have statistical characterizations, and in principle they can all help identify genes in long otherwise unannotated DNA sequence segments. To get an idea of the magnitude of the task with the human genome, consider the following facts about human gene sequences [5]: the coding regions comprise about 1.5% of the entire genome; the average gene length is about 27,000 bp (base pair); the average total coding region is 1,340 bp; and the average intron length is about 3,300 bp. Further, only about 8% of genes have a single exon. We see that the information in human genes is very dispersed along the genome, and that in general the parts of primary interest, the coding exons, are a relatively small fraction of the gene, on average about $\frac{1}{20}$.

8. Generalized HMMs for finding genes

The HMMs which are effective in finding genes are the generalized HMMs (GHMMs) described in section 5. above. Space does not permit our giving an adequate description here, so we simply outline the architecture of Genscan [1], one of the most widely used human gene finders. States represent the gene features we mentioned above: exon, intron, and of course intergenic region, and a variety of other features (promotor, untranslated region, polyA site, and so on). Output observations embody state-dependent nucleotide composition, dependence, and specific signal features (such as stop codons). In a GHMM the state duration needs to be modelled, as well as two other important features of genes in DNA: the reading frame, which corresponds to the triples along the mRNA sequence which are sequentially translated, and the strand, as DNA is double stranded, and genes can be on either strand, i.e. they can point in either direction. These features can be seen in Figure 4, which was kindly supplied by Lior Pachter.

The output of a GHMM genefinder after processing a genomic segment is broadly similar to that from a profile HMM after processing an amino acid sequence: the most probable state sequence given an observation sequence is a best gene annotation of that sequence, and a variety of probabilities can be calculated to indicate the support in the observation sequence for various specific gene features.

9. Comparative sequence analysis using HMMs

The large number of sequenced genomes now available, and the observation that functionally important regions are evolutionarily conserved, has led to efforts to incorporate conservation into the models and methods of biological sequence analysis. Pair HMMs were introduced in [2] as a way of including alignment problems under the HMM framework, and recently [4] they were combined with GHMMs (forming GPHMMs) to carry out alignment and gene finding with homologous segments of the mouse and human genomes. Use of the program SLAM on the whole
mouse genome (http://bio.math.berkeley.edu/slam/mouse/) demonstrated the value of GPHMMs in this context.

10. Challenges in biological sequence analysis

The first challenge is to understand the biology well enough to begin biological sequence analysis. This part will frequently involve collaborations with biologists. With HMMs, GHMMs and GPHMMs, designing the underlying architecture, and carrying out the modelling for the components parts, e.g. for splice sites in gene-finding GHMM is perhaps the next major challenge. Undoubtedly the hardest and most important task of all is the implementation: coding up the algorithms and making it all work with error-prone and incomplete sequence data. Finally, it is usually a real challenge to find good data sets for calibrating and evaluating the algorithms, and for carrying out studies of competing algorithms.

For a recent example of this process, which is a model of its kind, see [3]. There an HMM is presented for the so-called $\sigma^4$ recognition sites, which involve two DNA motifs separated by a variable number of base pairs. In addition to the examples mentioned so far, there are many more HMMs in the bioinformatics literature, see p. 79 of [2] for ones published before 1998.

11. Closing remarks
In this short survey of biological sequence analysis, I have simply touched on some of the major ideas. A much more comprehensive treatment of material covered here can be found in the book [2], whose title not coincidentally is the same as that of this paper. Many important ideas from biological sequence analysis have not been mentioned here, including molecular evolution and phylogenetic inference, and the use of stochastic context-free grammars, a form of generalization of HMMs suited to the analysis of RNA sequence data.

At this Congress I have talked (and am now writing) on the research of others, in an area in which my own contributions have been negligible. I chose to do so upon being honoured by the invitation to speak at this Congress because I believe this topic – HMMs – to be one of the great success stories of applying mathematics to bioinformatics. In my view it is the one most worthy of a wider mathematical audience. I hope that the fact that there are many others better suited than me to speak on this topic will not prevent readers from appreciating it and following it up through the bibliography.

I owe what understanding I have of this field to collaborations and discussions with a number of people, and I would like to acknowledge them here. Firstly, Tony Wirth, Simon Cawley and Mauro Delorenzi, with whom I have worked on HHMMs. Next, it has been an honour and pleasure to observe from close by the development of SLAM, by Simon Cawley, Lior Pachter and Marina Alexandersson. Finally I’d like to thank Xiaoyue Zhou and Ken Simpson for their kind help to me when I was preparing my talk and this paper.

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