Identifying Statistical Dependence in Genomic Sequences via Mutual Information Estimates[*]

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Abstract

Questions of understanding and quantifying the representation and amount of information in organisms have become a central part of biological research, as they potentially hold the key to fundamental advances. In this paper, we demonstrate the use of information-theoretic tools for the task of identifying segments of biomolecules (DNA or RNA) that are statistically correlated. We develop a precise and reliable methodology, based on the notion of mutual information, for finding and extracting statistical as well as structural dependencies. A simple threshold function is defined, and its use in quantifying the level of significance of dependencies between biological segments is explored. These tools are used in two specific applications. First, for the identification of correlations between different parts of the maize zmSRp32 gene. There, we find significant dependencies between the 5' untranslated region in zmSRp32 and its alternatively spliced exons. This observation may indicate the presence of as-yet unknown alternative splicing mechanisms or structural scaffolds. Second, using data from the FBI's Combined DNA Index System (CODIS), we demonstrate that our approach is particularly well suited for the problem of discovering short tandem repeats, an application of importance in genetic profiling.

Key Words: sequence analysis, statistical correlation, statistical significance, mutual information, exon, intron, alternative splicing, untranslated regions, error correction, tandem repeats.

[*]This research was supported in part by the NSF Grants CCR-0208709, CCF-0513636, and DMS-0503742, and the NIH Grant R01 GM068959-01.

1 Introduction

Questions of quantification, representation, and description of the overall flow of information in biosystems are of central importance in the life sciences. In this paper, we develop statistical tools based on information-theoretic ideas, and demonstrate their use in identifying informative parts in biomolecules. Specifically, our goal is to detect statistically dependent segments of biosequences, hoping to reveal potentially important biological phenomena. It is well-known [6, 19, 21] that various parts of biomolecules, such as DNA, RNA, and proteins, are significantly (statistically) correlated, although formal measures and techniques for quantifying these correlations are topics of current investigation. The biological implications of these correlations are deep, and they themselves remain unresolved. For example, statistical dependencies between *exons* carrying protein coding sequences and noncoding *introns* may indicate the existence of as-yet unknown error correction mechanisms or structural scaffolds. Thus motivated, we propose to develop precise and reliable methodologies for quantifying and identifying such dependencies, based on the information-theoretic notion of *mutual information*.

Biomolecules store information in the form of monomer strings such as deoxyribonucleotides, ribonucleotides, and amino acids. As a result of numerous genome and protein sequencing efforts, vast amounts of sequence data is now available for computational analysis. While basic tools such as BLAST provide powerful computational engines for identification of conserved sequence motifs, they are less suitable for detecting potential hidden correlations without experimental precedence (higher-order substitutions).

The application of analytic methods for finding regions of statistical dependence through mutual information has been illustrated through a comparative analysis of the 5' untranslated regions of DNA coding sequences [15]. It has been known that eukaryotic translational initiation requires the consensus sequence around the start codon defined as the Kozak's motif [11]. By screening at least 500 sequences, an unexpected correlation between positions -2 and -1 of the Kozak's sequence was observed, thus implying a novel translational initiation signal for eukaryotic genes. This pattern was discovered using mutual information, and not detected by analyzing single-nucleotide conservation. In other relevant work, neighbor-dependent substitution matrices were applied to estimate the average mutual information content of the core promoter regions from five different organisms [16, 17]. Such comparative analyses verified the importance of TATA-boxes and transcriptional initiation. A similar methodology elucidated patterns of sequence conservation at the 3' untranslated regions of orthologous genes from human, mouse, and rat genomes [20], making them potential targets for experimental verification of hidden functional signals.

In a different kind of application, statistical dependence techniques find important applications in the analysis of gene expression data. Typically, the basic underlying assumption in such analyses is that genes expressed similarly under divergent conditions share functional domains of biological activity. Establishing dependency or potential relationships between sets of genes from their expression profiles holds the key to the identification of novel functional elements. Statistical approaches to estimation of mutual information from gene expression datasets have been investigated in [21].

Protein engineering is another important area where statistical dependency tools are utilized. Reliable predictions of protein secondary structures based on long-range dependencies may enhance functional characterizations of proteins [2]. Since secondary structures are determined by both short- and long-range interactions between single amino acids, the application of comparative statistical tools based on consensus sequence algorithms or short amino acid sequences centered on the prediction sites, is far from optimal. Analyses that incorporate mutual information estimates may provide more accurate predictions.

In this work we focus on developing reliable and precise information-theoretic methods for determining whether two biosequences are likely to be statistically dependent. Another motivating factor for this project, which is more closely related to ideas from information theory, is the question of determining whether there are error correction mechanisms built into large molecules, as argued by Battail; see [3] and the references therein. We choose to work with protein coding exons and non-coding introns. While exons are well conserved parts of DNA, introns have much greater variability. They are dispersed on strings of biopolymers and still they have to be precisely identified in order to produce biologically relevant information. It seems that there is no external source of information but the structure of RNA molecules themselves to generate functional templates for protein synthesis. Determining potential mutual relationships between exons and introns may justify additional search for still unknown factors affecting RNA processing.

The complexity and importance of the RNA processing system is emphasized by the largely unexplained mechanisms of alternative splicing, which provide a source of substantial diversity in gene products. The same sequence may be recognized as an exon or an intron, depending on a broader context of splicing reactions. The information that is required for the selection of a particular segment of RNA molecules is very likely embedded into either exons or introns, or both. Again, it seems that the splicing outcome is determined by structural information carried by RNA molecules themselves, unless the fundamental dogma of biology (the unidirectional flow of information from DNA to proteins) is to be questioned.

Finally, the constant evolution of genomes introduces certain polymorphisms, such as *tandem repeats*, which are an important component of genetic profiling applications. We also study these forms of statistical dependencies in biological sequences using mutual information.

In Section 2 we develop some theoretical background, and we derive a threshold function for testing statistical significance. This function admits a dual interpretation either as the classical log-likelihood ratio from hypothesis testing, or as the "empirical mutual information."

Section 3 contains our experimental results: In Section 3.1 we present our empirical findings on the problem of detecting statistical dependency between different parts in a DNA sequence. Extensive numerical experiments were carried out on certain regions of the maize zmSRp32 gene [7], which is functionally homologous to the human ASF/SF2 alternative splicing factor. The efficiency of the empirical mutual information in this context is demonstrated. Moreover, our findings suggest the existence of a biological connection between the 5' untranslated region in zmSRp32 and its alternatively spliced exons.

Finally, in Section 3.2 we show how the empirical mutual information can be utilized in the difficult problem of searching DNA sequences for short tandem repeats (STRs), an important task in genetic profiling. We extend the simple hypothesis test of the previous sections to a methodology for testing a DNA string against different "probe" sequences, in order to detect STRs both accurately and efficiently. Experimental results on DNA sequences from the FBI's Combined DNA Index System (CODIS) are presented, showing that the empirical mutual information can be a powerful tool in this context as well.

2 Theoretical Background

In this section we outline the theoretical basis for the mutual information estimators we will later apply to biological sequences.

Suppose we have two strings of unequal lengths,

$$\begin{aligned} X_1^n &= X_1, X_2, \ldots, X_n && (1) \\ Y_1^M &= Y_1, Y_2, Y_3, \ldots\ldots\ldots, Y_M\,, && (2) \end{aligned}$$

where $M \geq n$, taking values in a common finite alphabet A. In most of our experiments, M is significantly larger than n; typical values of interest are $n \approx 80$ and $M \approx 300$. Our main goal is to determine whether or not there is some form of statistical dependence between them. Specifically, we assume that the string X_1^n consists of independent and identically distributed (i.i.d.) random variables X_i with common distribution $P(x)$ on A, and that the random variables Y_i are also i.i.d. with a possibly different distribution $Q(y)$. Let $\{W(y|x)\}$ be a family of conditional distributions, or "channel," with the property that, when the input distribution is P, the output has distribution Q, that is, $\sum_{x\in A} P(x)W(y|x) = Q(y)$, for all y. We wish to differentiate between the following two scenarios:

(I) Independence. X_1^n and Y_1^M are independent.

(II) Dependence. First X_1^n is generated. Then an index $J \in \{1, 2, \ldots, M-n+1\}$ is chosen in an arbitrary way, and Y_J^{J+n-1} is generated as the output of the discrete memoryless channel W with input X_1^n; that is, for each $j = 1, 2, \ldots, n$, the conditional distribution of Y_{j+J-1} given X_1^n is $W(y|X_j)$. Finally the rest of the Y_i's are generated i.i.d. according to Q. [To avoid the trivial case where both scenarios are identical, we assume that the rows of W are not all equal to Q so that in the second scenario X_1^n and Y_J^{J+n-1} are actually *not* independent.]

It is important at this point to note that, although neither of these two cases is biologically realistic as a description of the elements in a genomic sequence, it turns out that this set of assumptions provides a good operational starting point: The experimental results reported in Section 3 clearly indicate that, in practice, the resulting statistical methods obtained under the present assumptions can provide accurate and biologically relevant information.

To distinguish between *(I)* and *(II)*, we look at every possible alignment of X_1^n with Y_1^M, and we estimate the mutual information between them. Recall that for two random variables X, Y with marginal distributions $P(x), Q(y)$, respectively, and joint distribution $V(x, y)$, the mutual information between X and Y is defined as,

$$I(X;Y) = \sum_{x,y\in A} V(x,y) \log \frac{V(x,y)}{P(x)Q(y)}. \qquad (3)$$

Recall also [5] that $I(X;Y)$ is always nonnegative, and it equals zero if and only if X and Y are independent. The logarithms above and throughout the paper are taken to base 2, $\log = \log_2$, so that $I(X;Y)$ can be interpreted as the number of bits of information that each of these two random variables carries about the other; cf. [5].

In order to distinguish between the two scenarios above, we compute the empirical mutual information between X_1^n and each contiguous substring of Y_1^M of length n: For each $j = 1, 2, \ldots, M-n+1$, let $\hat{p}_j(x,y)$ denote the joint empirical distribution of (X_1^n, Y_j^{j+n-1}), i.e., let $\hat{p}_j(x,y)$ be the proportion of the n positions in $(X_1, Y_j), (X_2, Y_{j+1}), \ldots, (X_n, Y_{j+n-1})$ where (X_i, Y_{j+i-1}) equals (x, y). Similarly, let $\hat{p}(x)$ and $\hat{q}_j(y)$ denote the empirical distributions of

X_1^n and Y_j^{j+n-1}, respectively. We define the empirical (per-symbol) mutual information $\hat{I}_j(n)$ between X_1^n and Y_j^{j+n-1} by applying (3) to the empirical instead of the true distributions, so that,

$$\hat{I}_j(n) = \sum_{x,y\in A} \hat{p}_j(x,y) \log \frac{\hat{p}_j(x,y)}{\hat{p}(x)\hat{q}_j(y)}. \tag{4}$$

The law of large numbers implies that, as $n \to \infty$, we have $\hat{p}(x) \to P(x)$, $\hat{q}_j(y) \to Q(x)$, and $\hat{p}_j(x,y)$ converges to the true joint distribution of X, Y.

Clearly, this implies that in scenario *(I)*, where X_1^n and Y_1^n are independent, $\hat{I}_j(n) \to 0$, for any fixed j, as $n \to \infty$. On the other hand in scenario *(II)*, $\hat{I}_J(n)$ converges to $I(X;Y) > 0$ where the two random variables X, Y are such that X has distribution P and the conditional distribution of Y given $X = x$ is $W(y|x)$.

2.1 An Independence Test Based on Mutual Information

We propose to use the following simple *test* for detecting dependence between X_1^n and Y_1^M. Choose and fix a threshold $\theta > 0$, and compute the empirical mutual information $\hat{I}_j(n)$ between X_1^n and each contiguous substring Y_j^{j+n-1} of length n from Y_1^M. If $\hat{I}_j(n)$ is larger than θ for some j, declare that the strings X_1^n and Y_j^{j+n-1} are *dependent*; otherwise, declare that they are *independent.*

Before examining the issue of selecting the value of the threshold θ, we note that this statistic is identical to the (normalized) log-likelihood ratio between the above two hypotheses. To see this, observe that, expanding the definition of $\hat{p}_j(x,y)$ in $\hat{I}_j(n)$, we can simply rewrite,

$$\begin{aligned}
\hat{I}_j(n) &= \sum_{x,y\in A} \frac{1}{n}\sum_{i=1}^{n} \mathbb{I}_{\{(X_i,Y_{j+i-1})\}}(x,y) \log \frac{\hat{p}_j(x,y)}{\hat{p}(x)\hat{q}_j(y)} \\
&= \frac{1}{n}\sum_{i=1}^{n}\sum_{x,y\in A} \mathbb{I}_{\{(X_i,Y_{j+i-1})\}}(x,y) \log \frac{\hat{p}_j(x,y)}{\hat{p}(x)\hat{q}_j(y)},
\end{aligned}$$

where the indicator function $\mathbb{I}_{\{(X_i,Y_{j+i-1})\}}(x,y)$ equals 1 if $(X_i, Y_{j+i-1}) = (x,y)$, and it is equal to zero otherwise. Then,

$$\begin{aligned}
\hat{I}_j(n) &= \frac{1}{n}\sum_{i=1}^{n} \log \frac{\hat{p}_j(X_i,Y_{j+i-1})}{\hat{p}(X_i)\hat{q}_j(Y_{j+i-1})} \\
&= \frac{1}{n}\log\left[\frac{\prod_{i=1}^n \hat{p}_j(X_i,Y_{j+i-1})}{\prod_{i=1}^n \hat{p}(X_i)\hat{q}_j(Y_{j+i-1})}\right],
\end{aligned} \tag{5}$$

which is exactly the normalized logarithm of the ratio between the joint empirical likelihood $\prod_{i=1}^n \hat{p}_j(X_i, Y_{j+i-1})$ of the two strings, and the product of their empirical marginal likelihoods $[\prod_{i=1}^n \hat{p}(X_i)][\prod_{i=1}^n \hat{q}_j(Y_{j+i-1})]$.

2.2 Probabilities of Error

There are two kinds of errors this test can make: Declaring that two strings are dependent when they are not, and vice versa. The actual probabilities of these two types of errors depend on the distribution of the statistic $\hat{I}_j(n)$. Since this distribution is independent of j, we take

$j = 1$ and write $I(n)$ for the normalized log-likelihood ratio $\hat{I}_1(n)$. The next two subsections present some classical asymptotics for $\hat{I}_1(n)$.

Scenario (I): Independence. We already noted that in this case $I(n)$ converges to zero as $n \to \infty$, and below we shall see that this convergence takes place at a rate of approximately $1/n$. Specifically, $I(n) \to 0$ with probability one, and a standard application of the multivariate central limit theorem for the joint empirical distribution $\hat{p}_j$ shows that $nI(n)$ converges in distribution to a (scaled) χ^2 random variable. This a classical result in statistics [12, 18], and, in the present context, it was rederived by Hagenauer et al. [9, 8]. We have,

$$(2\ln 2)nI(n) \xrightarrow{\mathcal{D}} Z \sim \chi^2((|A|-1)^2),$$

where Z has a χ^2 distribution with $k = (|A|-1)^2$ degrees of freedom, and where $|A|$ denotes the size of the data alphabet.

Therefore, for a fixed threshold $\theta > 0$ and large n, we can estimate the probability of error as,

$$\begin{aligned} P_{e,1} &= \Pr\{\text{declare dependence} \,|\, \text{independent strings}\} \\ &= \Pr\{I(n) > \theta \,|\, \text{independent strings}\} \\ &\approx \Pr\{Z > (2\ln 2)\theta n\}, \end{aligned} \tag{6}$$

where Z is as before. Therefore, for large n the error probability $P_{e,1}$ decays like the tail of the χ^2 distribution function,

$$P_{e,1} \approx 1 - \frac{\gamma(k, (\theta \ln 2)n)}{\Gamma(k)},$$

where $k = \frac{(|A|-1)^2}{2}$, and Γ, γ denote the Gamma function and the incomplete Gamma function, respectively. Although this is fairly implicit, we know that the tail of the χ^2 distribution decays like $e^{-x/2}$ as $x \to \infty$, therefore,

$$P_{e,1} \approx \exp\Big\{ -(\theta \ln 2)n \Big\}, \tag{7}$$

where this approximation is to first order in the exponent.

Scenario (II): Dependence. In this case the asymptotic behavior of the test statistic $I(n)$ is somewhat different. Suppose as before that the random variables X_1^n are i.i.d. with distribution P, and that the conditional distribution of each Y_i given X_1^n is $W(y|X_i)$, for some fixed family of conditional distributions $W(y|x)$; this makes the random variables Y_1^n i.i.d. with distribution Q.

We mentioned in the last section that, under the second scenario, $I(n)$ converges to the true underlying value $I = I(X;Y)$ of the mutual information, but, as we show below, the rate of this convergence is slower than the $1/n$ rate of scenario *(I)*: Here, $I(n) \to I$ with probability one, but only at rate $1/\sqrt{n}$, in that, $\sqrt{n}[I(n) - I]$ converges in distribution to a Gaussian,

$$\sqrt{n}[I(n) - I] \xrightarrow{\mathcal{D}} V \sim N(0, \sigma^2), \tag{8}$$

where the resulting variance σ^2 is given by,

$$\sigma^2 = \mathrm{Var}\Big(\log\frac{W(Y|X)}{Q(Y)}\Big) = \sum_{x,y\in A} P(x)W(y|x)\Big(\log\frac{W(y|x)}{Q(y)} - I\Big)^2.$$

[An outline of the proof of (8) is given below.]

Therefore, for any fixed threshold $\theta < I$ and large n, the probability of error satisfies,

$$\begin{aligned} P_{e,2} &= \Pr\{\text{declare independence}\,|\,W\text{-dependent strings}\} \\ &= \Pr\{I(n) \le \theta\,|\,W\text{-dependent strings}\} \\ &\approx \Pr\{V \le [\theta - I]\sqrt{n}\} \\ &\approx \exp\Big\{-\frac{(I-\theta)^2}{2\sigma^2}\,n\Big\}, \end{aligned} \tag{9}$$

where the last approximation sign indicates equality to first order in the exponent. Thus, despite the fact that $I(n)$ converges at different speeds in the two scenarios, both error probabilities $P_{e,1}$ and $P_{e,2}$ decay exponentially with the sample size n.

To see why (8) holds it is convenient to use the alternative expression for $I(n)$ given in (5). Using this, and recalling that $I(n) = \hat{I}_1(n)$, we obtain,

$$\sqrt{n}[I(n) - I] = \sqrt{n}\Big[\frac{1}{n}\sum_{i=1}^{n}\log\frac{\hat{p}_1(X_i,Y_i)}{\hat{p}(X_i)\hat{q}_1(Y_i)} - I\Big].$$

Since the empirical distributions converge to the corresponding true distributions, for large n it is straightforward to justify the approximation,

$$\sqrt{n}[I(n) - I] \approx \frac{1}{\sqrt{n}}\Big[\sum_{i=1}^{n}\log\frac{P(X_i)W(Y_i|X_i)}{P(X_i)Q(Y_i)} - I\Big]. \tag{10}$$

The fact that this indeed converges in distribution to a $N(0,\sigma^2)$, as $n \to \infty$, easily follows from the central limit theorem, upon noting that the mean of the logarithm in (10) equals I and its variance is σ^2.

Discussion. From the above analysis it follows that, in order for both probabilities of error to decay to zero for large n (so that we rule out false positives as well as making sure that no dependent segments are overlooked) the threshold θ needs to be strictly between 0 and $I = I(X;Y)$. For that, we need to have some prior information about the value of I, i.e., of the level of dependence we are looking for. If the value of I were actually known and a fixed threshold $\theta \in (0, I)$ was chosen independent of n, then both probabilities of error would decay exponentially fast, but with typically very different exponents,

$$P_{e,1} \approx \exp\{-(\theta\ln 2)\,n\} \ \text{ and } \ P_{e,2} \approx \exp\Big\{-\Big(\frac{I-\theta}{\sqrt{2}\sigma}\Big)^2 n\Big\};$$

recall the expressions in (7) and (9). Clearly, balancing the two exponents also requires knowledge of the value of σ^2 in the case when the two strings are dependent, which, in turn, requires full knowledge of the marginal distribution P and the channel W. Of course this is unreasonable, since we cannot specify in advance the exact kind and level of dependence we are actually trying to detect in the data.

A practical (and standard) approach is as follows: Since the probability of error of the first kind $P_{1,e}$ only depends on θ (at least for large n), and since in practice declaring false positives is much more undesirable than overlooking potential dependence, in our experiments we decide on an acceptably small false-positive probability ϵ, and then select θ based on the above approximation, by setting $P_{e,1} \approx \epsilon$ in (6).

3 Experimental Results

In this section we apply the mutual information test described above to biological data. First we show that it can be used effectively to identify statistical dependence between regions of the maize zmSRp32 gene that may be involved in alternative processing (splicing) of pre-mRNA transcripts. Then we show how the same methodology can be easily adapted to the problem of identifying tandem repeats. We present experimental results on DNA sequences from the FBI's Combined DNA Index System (CODIS), which clearly indicate that the empirical mutual information can be a powerful tool for this computationally intensive task.

3.1 Detecting DNA Sequence Dependencies

All of our experiments were performed on the maize zmSRp32 gene [7]. This gene belongs to a group of genes that are functionally homologous to the human ASF/SF2 alternative splicing factor. Interestingly, these genes encode alternative splicing factors in maize and yet themselves are also alternatively spliced. The gene zmSRp32 is coded by 4735 nucleotides and has four alternative splicing variants. Two of these four variants are due to different splicings of this gene, between positions 1–369 and 3243–4220, respectively, as shown in Figure 1. The results given here are primarily from experiments on these segments of zmSRp32.

In order to understand and quantify the amount of correlation between different parts of this gene, we computed the mutual information between all functional elements including exons, introns, and the 5' untranslated region. As before, we denote the shorter sequence of length n by $X_1^n = (X_1, X_2, \ldots, X_n)$ and the longer one of length M by $Y_1^M = (Y_1, Y_2, \ldots, Y_M)$. We apply the simple mutual information estimator $\hat{I}_j(n)$ defined in (4) to estimate the mutual information between X_1^n and Y_j^{j+n-1} for each $j = 1, 2, \ldots, M-n+1$, and we plot the "dependency graph" of $\hat{I}_j = \hat{I}_j(n)$ versus j; see Figure 2. The threshold θ is computed according to (6), by setting ϵ, the probability of false positives, equal to 0.001; it is represented by a (red) straight horizontal line in the figures.

In order to "amplify" the effects of regions of potential dependency in various segments of the zmSRp32 gene, we computed the mutual information estimates $\hat{I}_j$ on the original strings over the regular four-letter alphabet $\{A, C, G, T\}$, as well as on transformed versions of the strings where pairs of letters were grouped together, using either the Watson-Crick pair $\{AT, CG\}$ or the purine-pyrimidine pair $\{AG, CT\}$. In our results we observed that such groupings are often helpful in identifying dependency; this is clearly illustrated by the estimates shown in Figures 2 and 3. Sometimes the $\{AT, CG\}$ pair produces better results, while in other cases the purine-pyrimidine pair finds new dependencies.

Figure 2 strongly suggests that there is significant dependence between the bases in positions 1–369 and certain substrings of the bases in positions 3243–4220. While the 1–369 region contains the 5' untranslated sequences, an intron, and the first protein coding exon, the 3243–4220 sequence encodes an intron that undergoes alternative splicing. After narrowing down

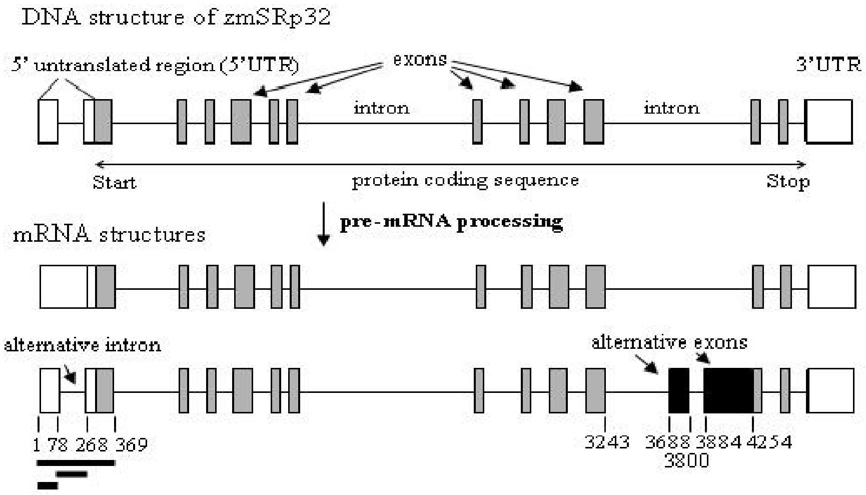
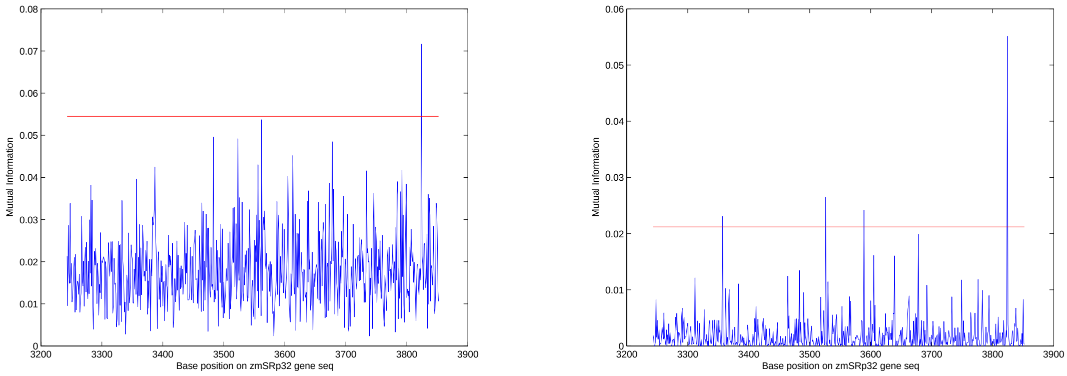


Figure 1: Alternative splicings of the zmSRp32 gene in maize. The gene consists of a number of exons (shaded boxes) and introns (lines) flanked by the 5' and 3' untranslated regions (white boxes). RNA transcripts (pre-mRNA) are processed to yield mRNA molecules used as templates for protein synthesis. Alternative pre-mRNA splicing generates different mRNA templates from the same transcripts, by selecting either alternative exons or alternative introns. The regions discussed in the text are identified by indices corresponding to the nucleotide position in the original DNA sequence.



(a)

(b)

Figure 2: Estimated mutual information between the exon located between bases 1–369 and each contiguous subsequence of length 369 in the intron between bases 3243–4220. The estimates were computed both for the original sequences in the standard four-letter alphabet $\{A, C, G, T\}$ (shown in (a)), as well as for the corresponding transformed sequences for the two-letter purine/pyrimidine grouping $\{AG, CT\}$ (shown in (b)).

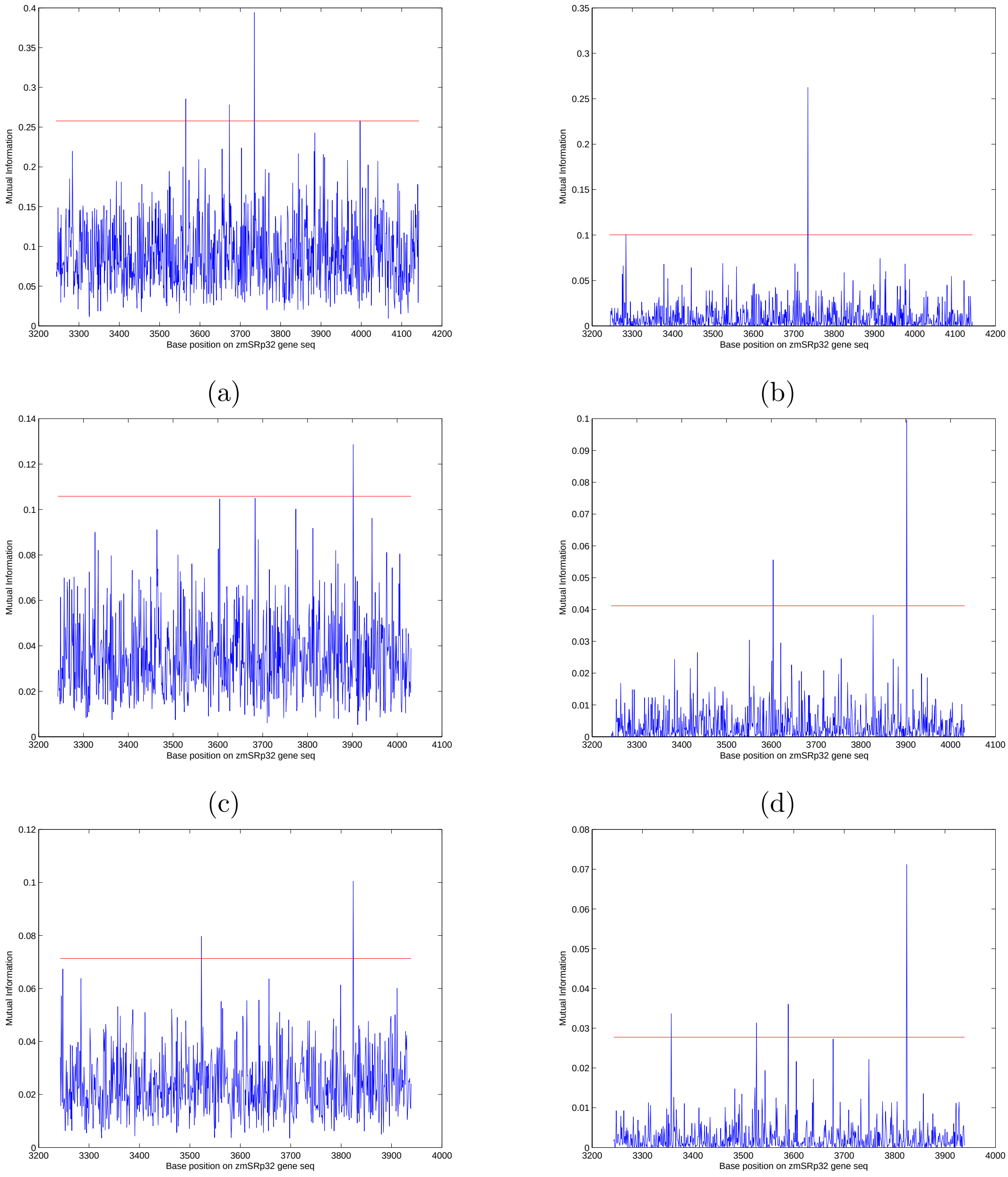
the mutual information calculations to the 5' untranslated region (5'UTR) in positions 1–78 and the 5'UTR intron in positions 78–268, we found that the initially identified dependency was still present; see Figure 3. A close inspection of the resulting mutual information graphs indicates that the dependency is restricted to the alternative exons embedded into the intron sequences, in positions 3688–3800 and 3884–4254.

These findings suggest that there might be a deeper connection between the 5'UTR DNA sequences and the DNA sequences that undergo alternative splicing. The UTRs are multifunctional genetic elements that control gene expression by determining mRNA stability and efficiency of mRNA translation. Like in the zmSRp32 maize gene, they can provide multiple alternatively spliced variants for more complex regulation of mRNA translation [10]. They also contain a number of regulatory motifs that may affect many aspects of mRNA metabolism. Our observations can therefore be interpreted as suggesting that the maize zmSRp32 5'UTR contains information that could be utilized in the process of alternative splicing, yet another important aspect of mRNA metabolism. The fact that the value of the empirical mutual information between 5'UTR and the DNA sequences that encode alternatively spliced elements is significantly greater than zero clearly points in that direction. Further experimental work could be carried out to verify the existence, and further explore the meaning, of these newly identified statistical dependencies.

We should note that there are many other sequence matching techniques, the most popular of which is probably the celebrated BLAST algorithm. BLAST's working principles are very different from those underlying our method. As a first step, BLAST searches a database of biological sequences for various small words found in the query string. It identifies sequences that are candidates for potential matches, and thus eliminates a huge portion of the database containing sequences unrelated to the query. In the second step, small word matches in every candidate sequence are extended by means of a Smith-Waterman-type local alignment algorithm. Finally, these extended local alignments are combined with some scoring schemes, and the highest scoring alignments obtained are returned. Therefore, BLAST requires a considerable fraction of exact matches to find sequences related to each other. However, our approach does not enforce any such requirements. For example, if two sequences do not have any exact matches at all, but the characters in one sequence are a character-wise encoding of the ones in the other sequence, then BLAST would fail to produce any significant matches (without corresponding substitution matrices), while our algorithm would detect a high degree of dependency. This is illustrated by the results in the following section, where the presence of certain repetitive patterns in Y_1^M is revealed through matching it to a "probe sequence" X_1^n which does *not* contain the repetitive pattern, but is "statistically similar" to the pattern sought.

3.2 Application to Tandem Repeats

Here we further explore the utility of the mutual information statistic, and we examine its performance on the problem of detecting Short Tandem Repeats (STRs) in genomic sequences. STRs, usually found in non-coding regions, are made of back-to-back repetitions of a sequence which is at least two bases long and generally shorter than 15 bases. The period of an STR is defined as the length of the repetition sequence in it. Owing to their short lengths, STRs survive mutations well, and can easily be amplified using PCR without producing erroneous data. Although there are many well-identified STRs in the human genome, interestingly, the number of repetitions at any specific locus varies significantly among individuals; that is, they



(a)

(b)

(c)

(d)

(e)

(f)

Figure 3: Dependency graph of $\hat{I}_j$ versus j for the zmSRp32 gene, using different alphabet groupings: In (a) and (b), we plot the estimated mutual information between the exon found between bases 1–78 and each subsequence of length 78 in the intron located between bases 3243–4220. Plot (a) shows estimates over the original four-letter alphabet $\{A, C, G, T\}$, and (b) shows the corresponding estimates over the Watson-Crick pairs $\{AT, CG\}$. Similarly, plots (c) and (d) contain the estimated mutual information between the intron located in bases 79–268 and all corresponding subsequences of the intron between bases 3243–4220. Plot (c) shows estimates over the original alphabet, and plot (d) over the two-letter purine/pyrimidine grouping $\{AG, CT\}$. Plots (e) and (f) show the estimated mutual information between the 5' untranslated region and all corresponding subsequences of the intron between bases 3243–4220, for the four-letter alphabet (in (e)), and for the two-letter purine/pyrimidine grouping $\{AG, CT\}$ (in (f)).

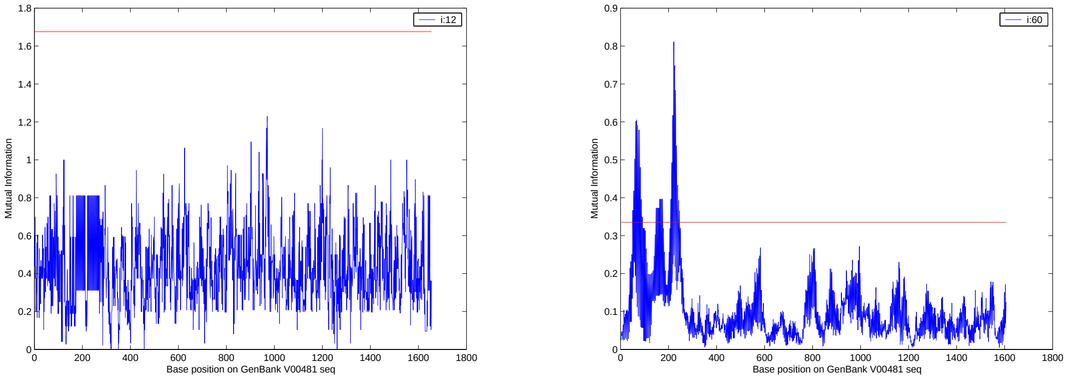
are *polymorphic* DNA fragments. These properties make STRs suitable tools for determining genetic profiles, and have become a prevalent method in forensic investigations. Long repetitive sequences have also been observed in genomic sequences, but have not gained as much attention since they cannot survive environmental degradation and do not produce high quality data from PCR analysis.

Several algorithms have been proposed for detecting STRs in long DNA strings with no prior knowledge about the size and the pattern of repetition. These algorithms are mostly based on pattern matching, and they all have high time-complexity. Finding short repetitions in a long sequence is a challenging problem. When the query string is a DNA segment that contains many insertions, deletions or substitutions due to mutations, the problem becomes even harder. Exact- and approximate-pattern matching algorithms need to be modified to account for these mutations, and this renders them complex and inefficient. To overcome these limitations, we propose a statistical approach using an adaptation of the method described in the previous sections.

In the United States, the FBI has decided on 13 loci to be used as the basis for genetic profile analysis, and they continue to be the standard in this area. To demonstrate how our approach can be used for STR detection, we chose to use sequences from the FBI's Combined DNA Index System (CODIS): The SE33 locus contained in the GenBank sequence V00481, and the VWA locus contained in the GenBank sequence M25858. The periods of STRs found in CODIS typically range from 2 to 4 bases, and do not exhibit enough variability to demonstrate how our approach would perform under divergent conditions. For this reason, we used the V00481 sequence as is, but on M25858 we artificially introduced an STR with period 11, by substituting bases 2821–2920 (where we know that there are no other repeating sequences) with 9 tandem repeats of $ACTTTGCCTAT$. We have also introduced base substitutions, deletions and insertions on our artificial STR to imitate mutations.

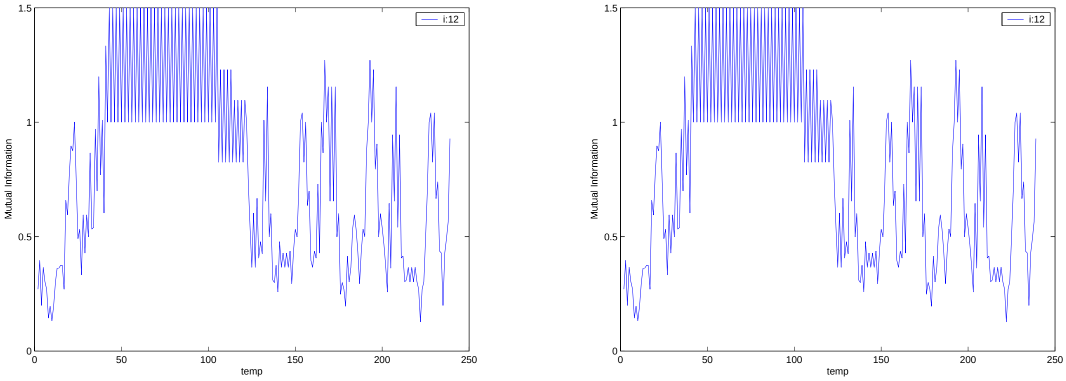
Let $Y_1^M = (Y_1, Y_2, \ldots, Y_M)$ denote the DNA sequence in which we are looking for STRs. The gist of our approach is simply to choose a *periodic* probe sequence of length n, say, $X_1^n = (X_1, X_2, \ldots, X_n)$ (typically much shorter than Y_1^M), and then to calculate the empirical mutual information $\hat{I}_j = \hat{I}_j(n)$ between X_1^n and each of its possible alignments with Y_1^M. In order to detect the presence of STRs, the values of the empirical mutual information in regions where STRs do appear should be significantly larger than zero, where "significantly" means larger than the corresponding estimates in ordinary DNA fragments containing no STRs. Obviously, the results will depend heavily on the exact form of the probe sequence. Therefore, it is critical to decide on the method for selecting: (a) the length, and (b) the exact contents of X_1^n. The length of X_1^n is crucial; if it is too short, then X_1^n itself is likely to appear often in Y_1^M, producing many large values of the empirical mutual information and making it hard to distinguish between STRs and ordinary sequences. Moreover, in that case there is little hope that the analysis of the previous section (which was carried out of long sequences X_1^n) will provide useful estimates for the probability of error. If, on the other hand, X_1^n is too long, then any alignment of the probe X_1^n with Y_1^M will likely also contain too many irrelevant base pairs. This will produce negligibly small mutual information estimates, again making impossible to detect STRs. These considerations are illustrated by the results in Figure 4.

As for the contents of the probe sequence X_1^n, the best choice would be to take a segment X_1^n containing an exact match to an STR present in Y_1^M. But in most of the interesting applications, this is of course unavailable to us. A "second best" choice might be a sequence X_1^n that contains a segment of the same "pattern" as the STR present in Y_1^M, where we say



(a) (b)

Figure 4: Dependency graph of the GenBank sequence $Y_1^M = V00481$, for a probe sequence X_1^n which is a repetition of $AGGT$, of length: (a) 12, or (b) 60. The sequence Y_1^M contains STRs that are repetitions of the pattern $AAAG$, in the following regions: (i) there is a repetition of $AAAG$ between bases 62–108; (ii) $AAAG$ is intervened by AG and $AAGG$ until base 138; (iii) again between 138–294 there are repetitions of $AAAG$, some of which are modified by insertions and substitutions. In (a) our probe is too short, and it is almost impossible to distinguish the SE33 locus from the rest. However, in (b) the location SE33 is singled out by the two big peaks in the mutual information estimates; the shorter peak between the two larger ones is due to the interventions described above. Note that the STRs were identified by a probe sequence that was a repetition of a pattern *different* from that of the repeating part of the STRs themselves, but of the same period.



(a) (b)

Figure 5: Dependency graph of the VWA locus contained in GenBank sequence M25858 for a probe sequence X_1^n with $n = 12$, which is a repetition of: (a) $TCTA$, an exactly matching probe, (b) $GTGC$, a completely different probe, but of the exact same "pattern." In both cases, we have chosen X_1^n to be long enough to suppress unrelated information. Note that the results in (a) and (b) are almost identical. The VWA locus contains an STR of $TCTA$ between positions 44–123. This STR is apparent in both dependency graphs by forming a periodic curve with high correlation.

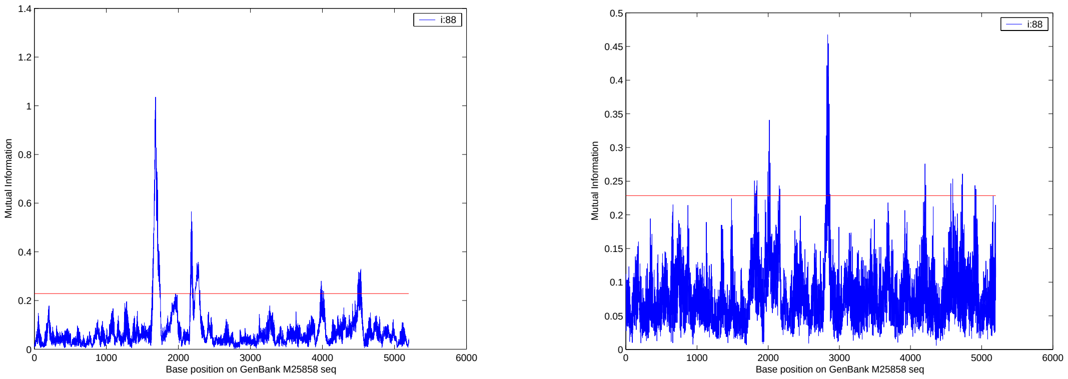
that two sequences have the same *pattern* if each one can be obtained from the other via a permutation of the letters in the alphabet; cf. [1, 14]. For example, $TCTA$ and $GTGC$ have the same pattern, whereas $TCTA$ and $CTAT$ do not (although the do have the same empirical distribution). For example, if X_1^n contains the exact same pattern as the periodic part of the STR to be detected, and $\tilde{X}_1^n$ has the same pattern as X_1^n, then, a priori, either choice should be equally effective at detecting the STR under consideration; see Figure 5. [This observation also shows that a single probe X_1^n may in fact be appropriate for locating more than a single STR; for example, STRs with the same pattern as X_1^n, as in Figure 5, or with the same period, as in Figure 4.] The problem with this choice is, again, that the exact patterns of STRs present in a DNA sequence are not available to us in advance, and we cannot expect all STRs in a given sequence to be of the same pattern.

Even though both of the above choices for X_1^n are usually not practically feasible, if the sequence Y_1^M is relatively short and contains a single STR whose contents are known, then either choice would produce high quality data, from which the STR contained in Y_1^M we can easily be detected; see Figure 5 for an illustration.

In practice, in addition to the fact that the contents of STRs are not known in advance, there is also the issue that in a long DNA sequence there are often many different STRs, and a unique probe will not match all of them exactly. But since STRs usually have a period between 2 and 15 bases, we can actually run our method for all possible choices of repetition sequences, and detect all STRs in the given query sequence Y_1^M. The number of possible probes X_1^n can be drastically by observing that (1) We only need one repeating sequence of each possible pattern; and (2) It suffices to only consider repetition patters whose period is prime. Note that, in view of the earlier discussion and the results shown in Figure 4, the period of the repeating part of X_1^n is likely to be more important than the actual contents. For example, if we were to apply our method for finding STRs in Y_1^M with a probe X_1^n whose period is 5 bases long, then many STRs with a period that is a multiple of 5 should peak in the dependency chart, thus allowing us to detect their approximate positions in Y_1^M. Clearly, probes that consist of very short repeats, such as $AAA\ldots\ldots$, should be avoided. The importance of choosing an X_1^n with the correct period is illustrated in Figure 6.

The results in Figures 4, 5 and 6 clearly indicate that the proposed methodology is very effective at detecting the presence of STRs, although at first glance it may appear that it cannot provide precise information about their start-end positions and their repeat sequences. But this final task can easily be accomplished by re-evaluating Y_1^M near the peak in the dependency graph, for example, by feeding the relevant parts separately into one of the standard string matching-based tandem repeat algorithms. Thus, our method can serve as an initial filtering step which, combined with an exact pattern matching algorithm, provides a very accurate and efficient method for the identification of STRs.

In terms of its practical implementation, note that our approach has a linear running time $O(M)$, where M is the length of Y_1^M. The empirical mutual information of course needs to be evaluated for every possible alignment of Y_1^M and X_1^n, with each such calculation done in $O(n)$ steps, where n is the length of X_1^n. But n is typically no longer than a few hundred bases, and, at least to first order, it can be considered constant. Also, repeating this process for all possible repeat periods does not affect the complexity of our method by much, since the number of such periods is quite small and can also be considered to be constant. And, as mentioned above, choosing probes X_1^n only containing repeating segments with a prime period, further improves the running time of our method.



(a)

(b)

Figure 6: In these charts we use the modified GenBank sequence M25858, which contains the VWA locus in CODIS between positions 1683–1762 and the artificial STR introduced by us at 2821–2920. The repeat sequence of the VWA locus is $TCTA$, and the repeat sequence of the artificial STR is $ACTTTGCCTAT$. In (a), the probe X_1^n has length $n = 88$ and consists of repetitions of $AGGT$. Here the repeating sequence of the VWA locus (which has period 4) is clearly indicated by the peak, whereas the artificial tandem repeat (which has period 11) does not show up in the results. The small peak around position 2100 is due to a very noisy STR again with a 4 base period. In (b), the probe X_1^n again has length $n = 88$, and it consists of repetitions of $CATAGTTCGGA$. This produces the opposite result: The artificial STR is clearly identified, but there is no indication of the STR present at the VWA locus.

We, therefore, conclude that: (a) the empirical mutual information appears in this case to be a very effective tool for detecting STRs; and (b) selecting the length and repetition period of the probe sequence X_1^n is crucial for identifying tandem repeats accurately.

4 Conclusions

Biological information is stored in the form of monomer strings composed of conserved biomolecular sequences. According to Manfred Eigen, “The differentiable characteristic of living systems is information. Information assures the controlled reproduction of all constituents, thereby ensuring conservation of viability.” Hoping to reveal novel, potentially important biological phenomena, we employ information-theoretic tools, especially the notion of mutual information, to detect statistically dependent segments of biosequences. The biological implications of the existance of such correlations are deep, and they themselves remain unresolved. The proposed approach may provide a powerful key to fundamental advances in understanding and quantifying biological information.

This work addresses two specific applications based on the proposed tools. From the experimental analysis carried out on regions of the maize zmSRp32 gene, our findings suggest the existence of a biological connection between the 5’ untranslated region in zmSRp32 and its alternatively spliced exons, potentially indicating the presence of novel alternative splicing mechanisms or structural scaffolds. Secondly, through extensive analysis of CODIS data, we show that our approach is particularly well suited for the problem of discovering short tandem repeats, an application of importance in genetic profiling studies.

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