PIntron: a Fast Method for Gene Structure Prediction via Maximal Pairings of a Pattern and a Text

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May 11, 2010

Abstract

Current computational methods for exon-intron structure prediction from a cluster of transcript (EST, mRNA) data do not exhibit the time and space efficiency necessary to process large clusters of over 20,000 ESTs and genes longer than 1Mb. Guaranteeing both accuracy and efficiency seems to be a computational goal quite far to be achieved, since accuracy is strictly related to exploiting the inherent redundancy of information present in a large cluster.

We propose a fast method for the problem that combines two ideas: a novel algorithm of proved small time complexity for computing spliced alignments of a transcript against a genome, and an efficient algorithm that exploits the inherent redundancy of information in a cluster of transcripts to select, among all possible factorizations of EST sequences, those allowing to infer splice site junctions that are highly confirmed by the input data. The EST alignment procedure is based on the construction of maximal embeddings that are sequences obtained from paths of a graph structure, called Embedding Graph, whose vertices are the maximal pairings of a genomic sequence T and an EST P. The procedure runs in time linear in the size of P, T and of the output.

PIntron, the software tool implementing our methodology, is able to process in a few seconds some critical genes that are not manageable by other gene structure prediction tools. At the same time, PIntron exhibits high accuracy (sensitivity and specificity) when compared with ENCODE data.

Detailed experimental data, additional results and PIntron software are available at http://www.algolab.eu/PIntron.

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1 Introduction

A key step in the post-transcriptional modification process is called splicing and consists in the excision of the intronic regions of the premature mRNA (pre-mRNA) while the exonic regions are then reconnected to re-form a single continuous molecule, the mature mRNA. A complex regulatory system mediates the splicing process which, under different conditions, may produce alternative mature mRNAs (also called transcript isoforms) starting from a single pre-mRNA molecule. Alternative Splicing (AS), i.e. the production of alternative transcripts from the same gene, is the main mechanism responsible for the expansion of the transcriptome (the set of transcripts generated by the genome of one organism) in eukaryotes and it is also involved in the onset of several diseases [7].

A great extent of work has been performed to solve two basic problems on AS: characterizing the exon-intron structure of a gene and finding the set of different transcript isoforms that are produced from the same gene. Some computational approaches to these crucial problems have been proposed; indeed good implementations are available [14, 17, 21, 16, 9, 8]. In this paper we focus on providing an algorithm – efficient from both a theoretical and an empirical point of view – to predict the exon-intron structure: a problem that is still unsolved by methods based on transcript data.

Indeed, it must be pointed out that few efforts have been done in the direction of providing a formal framework to design efficient algorithms for the general AS prediction problem. As a matter of fact, existing tools are not able to process efficiently genes that are huge or with a very large set of associated clusters of ESTs [8]. A basic reason of this fact is that combinatorial methods for the problem must combine two different steps: (1) to produce putative spliced alignments of ESTs against the gene region and (2) to use redundancy and the whole covering of the gene region provided by a cluster in the prediction process by selecting among putative spliced alignments of multiple ESTs [2] the ones that confirm the same gene structure. The two steps are much harder when combined. In fact the literature provides efficient solutions of the first step when solved independently from the second one. Finding an alignment of an EST sequence could be a hard task when more than one alignment can exist for the same input data and a primary goal is the choice of the alignment that integrates biological meaningfulness w.r.t. the whole gene structure. On the other side the second step is NP-hard [3] thus requiring efficient heuristics.

In this paper we show how to efficiently solve the integration of the above two steps. First we design a new fast algorithm for producing splicing alignments of EST sequences by exploiting a new combinatorial formulation of the problem. Afterwards, given the spliced alignments for each EST sequence of a gene, for each EST we extract a composition that is consistent with a putative exon-intron structure of the given gene, by applying the Minimum Factorization Agreement (MFA) approach to the data produced by our spliced alignment algorithm [3]. Indeed, the MFA approach provides an effective method to extract the compositions in such a way that the whole gene structure is derived (as an
EST sequence provides the information on a partial region of the whole gene).

2 System and Methods

Our new combinatorial method for exon-intron structure prediction can be summarized as a four-stage pipeline where we: (1) compute and implicitly represent of all the spliced alignments of a transcript sequence (EST or mRNA) against a genomic reference sequence; (2) filter all biologically meaningful spliced alignments; (3) reconcile the spliced alignments of a set of correlated transcript sequences into a consensus gene structure; (4) extract, classify and refine the resulting introns. In the following we describe the main idea on which the first two steps of our pipeline are built.

A basic ingredient of most computational approaches for gene structure prediction is aligning several ESTs against the reference genomic sequence \([15, 12, 22, 10, 20, 5]\) taking into account the effects of the excision of the intronic regions. Thus, when considering ESTs, a particular kind of alignment problem arises: the spliced sequence alignment. The spliced sequence alignment problem requires to compute, given a sequence \(S\) and a reference sequence \(T\), two sets \(F_S = \{f_1, \ldots, f_k\}\) and \(F_T = \{f'_1, \ldots, f'_k\}\) of strings such that \(S = f_1 \cdots f_k\), \(T = p f'_1 i_1 \cdots i_{k-1} f'_k s\), and for each \(i\), the edit distance between \(f_i\) and \(f'_i\) is small. The sequence of pairs \((f_i, f'_i)\) is called composition of \(S\) on \(T\), each factor \(f_i\) is called spliced sequence factor (or EST factor), and each \(f'_i\) is called genomic factor (or exon). Clearly, in the biological context, the sequence \(S\) is an EST or a mRNA sequence, while the reference sequence \(T\) is the genomic sequence of the locus of the gene where the EST comes from. Also allowing a small edit distance between two factors is justified by the fact that EST data contain mismatches (deletions and insertions) against the genome because of sequencing errors and polymorphisms.

Notice that allowing an approximate alignment between factors makes the spliced alignment problem computationally harder, especially when EST data and the genomic sequence are large. Moreover, multiple compositions can exist for the same input data and thus integrating biological meaningfulness is a primary goal.

One of the main ideas of our approach is that the small edit distance between a sequence factor and a genomic factor implies that there exist some perfectly conserved subfactors (called pairings) between those factors. The problem of combining together such pairings is simplified by the introduction of a new data structure called Embedding Graph which is a graph representing all spliced alignments. Building and querying the Embedding Graph is not trivial and is the main subject of our algorithmic investigation discussed in the next section.

The whole four-step pipeline method has been implemented and experimented. We designed our experimental analysis in two parts, according to the two goals that we wanted to achieve, namely to investigate the scalability of our implementation and to assess the quality of the results obtained. For each gene the associated human Unigene cluster has been processed by our spliced
alignment algorithm, together with the whole ENCODE region. Indeed, we have assessed the quality of the results by comparing the outputs computed by our implementation with the gene structure data provided by ENCODE which aimed at providing a reliable annotation of 1% human genome [19].

For the assessment of the prediction accuracy, we use two quality measures, sensitivity and specificity, commonly adopted for the evaluation of computational gene-structure prediction tools [11]. We call benchmark set $B$ and test set $I$ respectively the data retrieved from ENCODE and the results computed by our approach. Sensitivity is defined as the ratio $|B \cap I|/|B|$, while specificity $^1$ is defined as the ratio $|B \cap I|/|I|$. Notice that both ratios have values between 0 and 1, and that higher values correspond to higher similarity between $B$ and $I$. We will analyze the results produced by our pipeline according to two coordinates: the set of predicted introns and the set of predicted splice sites.

When considering the set of predicted introns, an intron $e \in B$ belongs to $B \cap I$ if there exists a prediction $i \in I$, perfectly matching $e$ both on the donor and the acceptor splice sites on the genomic sequence.

The first experiment has been run on a set of 112 fairly typical genes from 13 ENCODE regions with lengths ranging from 0.5Mb to 1.7Mb encompassing 98,064 transcripts (total length 63Mb), on which we have studied both the running times and the quality of the results. The second experiment, devoted to studying the scalability of our approach, has been performed on some hand-picked genes exhibiting a large genomic sequence or a large set of transcripts. More precisely, we have chosen 26 genes of which 11 are at least 1Mb long (on average about 848Kb), and 5 have more than 15,000 transcripts (on average more than 5000 transcripts). The complete list of regions and genes studied in both experiments, as well as the data supporting our analysis, is in the supplementary material.

3 Methods

3.1 Implicit Computation of Spliced Alignments

The first stage of our gene structure prediction method computes the set of all possible spliced alignments of an EST (or mRNA) sequence against the genomic sequence. In particular, the first stage computes an implicit compact representation of the spliced alignments, which is then used by the second stage to extract all biologically plausible alignments.

In our novel alignment method, we exploit a fundamental property of the notion of composition: the edit distance between each pair of corresponding factors is small. Therefore, there must exist some sufficiently long common substrings of the EST factors and the genomic factors. For example, a typical low-quality sequencing technology produces a 50bp-long exon with a 6% error rate. The worst scenario still allows for pairs of 11bp-long substrings that per-

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$^1$ We adopted the definition of specificity used when evaluating gene-structure prediction tools, even if not standard [1].
fectly match. Clearly, if the sequence of perfectly matching pairs of substrings is known, it is quite easy to reconstruct a possible alignment of the factors. We call the sequence of the occurrences of perfectly matching pairs an embedding of the EST sequence in the genomic sequence. Notice that there might be more than one embedding of the EST sequence \( P \) in the genomic sequence \( T \). We can compute efficiently all embeddings maintaining a graph whose vertices are all possible maximal pairings of \( P \) and \( T \). A maximal pairing of \( P \) and \( T \) generalizes the notion of maximal pair of a sequence \([13]\) and it provides the occurrence on \( P \) and \( T \) of a maximal common substring of \( P \) and \( T \). The vertex set \( V \) can be computed in time linear in the sizes of \( P \), \( T \), and \( V \). Edges of the graph are computed in time at most \( O(|V|^2) \).

According to the traditional notation, given a string \( S = s_1s_2\cdots s_q \), we denote with \(|S|\) its length and with \( S[i,j] \) the substring \( s_is_{i+1}\cdots s_j \). A fundamental notion is that of pairing of two strings. Given a pattern \( P \) and a text \( T \), a pairing of \( P \) and \( T \) is a triplet \((p,t,l)\) such that \( P[p,p+l-1] = T[t,t+l-1] \). In other words, a pairing \((p,t,l)\) represents a common substring \( x \) (or factor) of \( P \) and \( T \) of length \( l \) starting in positions \( p \) and \( t \) on \( P \) and \( T \) respectively. We call \( p \) and \( t \) the starting position on \( P \) and \( T \) respectively, \( p+l \) and \( t+l \) the ending position on \( P \) and \( T \) respectively, while \( l \) is the length of the pairing. When no ambiguities arise, the two strings \( P \) and \( T \) will be omitted when dealing with a pairing.

Let \( f \) be the common factor represented by a pairing \( v = (p,t,l) \). Notice that all triplets \((p+\delta_1,t+\delta_1,l-\delta_2)\), with \( 0 < \delta_1 < l \) and \( \delta_1 \leq \delta_2 \leq l - \delta_1 \) are also pairings. It is natural to define an order \( \preceq \) among pairings. Let \( v_1 = (p_1,t_1,l_1) \) and \( v_2 = (p_2,t_2,l_2) \) be two pairings, then \( v_1 \preceq v_2 \) iff \( p_2 \leq p_1 \leq p_1 + l_1 \leq p_2 + l_2 \) and \( p_1 - p_2 = t_1 - t_2 \). When \( v_1 \preceq v_2 \) we say that \( v_1 \) is a sub-pairing of \( v_2 \), or \( v_2 \) contains \( v_1 \). Moreover, \( v_1 \) is a prefix-pairing (suffix-pairing, resp.) of \( v_2 \) iff \( v_1 \preceq v_2 \) and the starting positions (the ending positions, resp.) of \( v_1 \) and \( v_2 \) on \( P \) and \( T \) are equal.

Based on the order relation \( \preceq \) we can define the concept of maximality of pairings. A pairing \( v \) is maximal if and only if it is neither a suffix-pairing nor a prefix-pairing of a distinct pairing \( v' \). In other words, \( v = (p,t,l) \) is maximal if and only if \( P[p-1] \neq T[t-1] \) and \( P[p+l] \neq T[t+l] \).

We recall that an embedding is a sequence of pairings. Therefore we can extend \( \preceq \) to an order between embeddings and, based on this, we derive the notion of maximal embeddings. Given two embeddings \( \varepsilon = \langle v_1,\ldots,v_n \rangle \) and \( \varepsilon' = \langle v'_1,\ldots,v'_m \rangle \), then \( \varepsilon \) is contained in \( \varepsilon' \) (in short \( \varepsilon \preceq \varepsilon' \)) if and only if for each \( v_i \) in \( \varepsilon \) there exists a pairing \( v'_j \) in \( \varepsilon' \) such that \( v_i \preceq v'_j \). Given the set \( \mathcal{E} \) of the embeddings of \( P \) in \( T \), we say that \( \varepsilon \in \mathcal{E} \) is maximal iff there does not exist \( \varepsilon' \in \mathcal{E} \), \( \varepsilon \neq \varepsilon' \), such that \( \varepsilon \preceq \varepsilon' \).

Not all embeddings induce a biologically meaningful composition. For example, an embedding constituted by several short pairings “scattered” along the genome cannot be considered a valid spliced alignment. A representative embedding is a maximal embedding \( \varepsilon = \langle v_1,\ldots,v_m \rangle \) such that \( l_i \geq \ell_E \) and \( p_{i+1} - p_i - l_i \leq \ell_D \), and either (i) \( t_{i+1} - t_i - p_{i+1} + p_i \leq \ell_D \) or (ii) \( t_{i+1} - t_i - p_{i+1} + p_i \geq \ell_I \) is true – only representative embeddings might induce
a biologically plausible composition. Intuitively, the parameter \( \ell_E \) is the minimum length of a pairing (in order to avoid the previous example), \( \ell_D \) regulates the maximum number of consecutive mismatches, and \( \ell_I \) represents the minimum length of a valid intron. For each composition such that every \( \ell_D \)-long substring of the EST sequence is aligned with the corresponding genomic factors with up to \( \frac{\ell_D}{\ell_E} \) errors, there exists (at least) one representative embedding inducing such a composition (if we choose carefully the values of the parameters). This fact roughly means that spliced alignments with error rate at most \( \frac{1}{\ell_E} \) can be recovered from some representative embeddings.

Therefore we propose the problem of finding all the representative embeddings of \( P \) in \( T \), formalized as the Representative Embedding Problem (RE), where we are given a pattern \( P \), a text \( T \), and three parameters \( \ell_E, \ell_D \) and \( \ell_I \). The goal is to compute the set \( \mathcal{E}_r \) of the representative embeddings of \( P \) in \( T \).

We are now able to introduce the embedding graph, which is our main device for tackling the RE problem.

**Definition 3.1 (Embedding Graph).** Given a pattern \( P \) and a text \( T \), the embedding graph of \( P \) in \( T \) is a directed graph \( G = (V,E) \) such that the vertex-set \( V \) is composed by the set of maximal pairings of \( P \) and \( T \) longer than \( \ell_E \), and two pairings \( v_1 = (p_1,t_1,l_1) \) and \( v_2 = (p_2,t_2,l_2) \) are connected by an edge \((v_1,v_2)\) if and only if: (i) \( p_2 - p_1 - l_1 \leq \ell_D \), and (ii) \( |t_2 - t_1 - p_2 + p_1| \leq \ell_D \) or \( t_2 - t_1 - p_2 + p_1 \geq \ell_I \).

Basically the above conditions ensure that if two maximal pairings \( v_1 \) and \( v_2 \) are connected by an edge in the embedding graph, then there exists a representative embedding \( \varepsilon \) in which a sub-pairing of \( v_1 \) and a sub-pairing of \( v_2 \) are consecutive. It is possible to prove that also the converse proposition is true: that is, if two pairings \( v'_1 \) and \( v'_2 \) are consecutive in a representative embedding \( \varepsilon \), then the maximal pairings \( v_1 \) and \( v_2 \) which contain \( v'_1 \) and \( v'_2 \), respectively, are connected by an edge in the embedding graph. The previous properties derive from the maximality of the representative embeddings and from the uniqueness of the maximal pairing containing a pairing which belongs to a representative embedding.

We designed an algorithm which builds the embedding graph of a pattern \( P \) and a text \( T \) in time \( O(|T| + |P| + |V|^2) \). The algorithm is composed by two steps: In the first step, the vertex-set \( V \) is computed by visiting the suffix-tree of the text \( T \). This step requires \( O(|T|) \) time for the suffix-tree construction and \( O(|P| + |V|) \) time for the computation of maximal pairings. In the second step, edges are then computed by checking the conditions of Definition 3.1 on each pair of maximal pairings, leading to a \( O(|V|^2) \) procedure. Since the number of maximal pairings is usually very small compared to the length of \( P \) and \( T \), the embedding graph construction procedure is efficient even on large patterns \( P \) and texts \( T \).
Figure 1: Possible configurations of relative positions of two maximal pairings $v, v'$ connected by an embedding graph edge. Each box represents a common maximal factor on $T$ (top) and $P$ (bottom) of a maximal pairing. Then $v, v'$ are represented by two boxes connected by dotted lines. Four possible cases are presented: (a) $v, v'$ overlap on both $T$ and $P$, (b) $v, v'$ overlap on $T$ but not on $P$, (c) $v, v'$ overlap on $P$ but not on $T$, and (d) $v, v'$ do not overlap neither on $T$ nor on $P$.

### 3.2 Extraction of Relevant Spliced Alignments

The next stage of our pipeline is devoted to analyzing and mining the embedding graph to compute the representative embeddings that also induce distinct biologically meaningful compositions. Given two pairings that are connected by an edge in the embedding graph, the corresponding factors might be overlapping in the text or in the pattern, leading to four different configurations that are depicted in Fig. 1.

Algorithm ComputeCompositions is a two-step procedure. Initially it extracts a subset of representative embeddings by performing a visit of the embedding graph. Then the algorithm computes the compositions by merging consecutive pairings that are separated by short gaps. Basic biological criteria (such as the recognition of canonical splice sites) are locally used, if possible, to resolve ambiguities in the exon-intron boundary determination. These boundaries can be changed during the last stage of the pipeline according to some refinement criteria which globally analyze the set of introns induced by the spliced alignments of all the transcripts.
Embedding graph visit. The first step of ComputeCompositions is a recursive visit of the embedding graph starting from a subset of vertices that we call extended sources. The visit of a vertex $v_k$ from the extended source $s$ constructs the set $E$ of biologically meaningful representative embeddings that are induced by the path $P = \langle s, v_1, \ldots, v_k \rangle$ traversed during the visit. Due to space constraints we are unable to provide the formal characterization of extended sources, as well as some technical details of the visit and a proof of the correctness of the step – each representative embedding has been examined, only the biologically meaningful ones have been added to set $E$, and the visits compute pairwise-distinct representative embeddings.

During the visit of vertex $v_k$, we examine each outgoing edge $(v_k, v_{k+1})$ and we “extend” each embedding $\varepsilon = \langle u_1, \ldots, u_k \rangle$ of $E$. How the extension is performed depends on the overlapping of the involved factors, i.e. it depends on the four possible configurations of relative positions between the last pairing $u_k$ of $\varepsilon$ and the new vertex $v_{k+1}$ that are depicted in Fig. 1. In the exposition of the four cases, let $u_k = (p_k, t_k, l_k)$ and $v_{k+1} = (p_{k+1}, t_{k+1}, l_{k+1})$.

Case (a). Factors $u_k$ and $v_{k+1}$ overlap on both $T$ and $P$. Two different sub-cases must be analyzed: If $|(t_{k+1} - t_k) - (p_{k+1} - p_k)| \leq \ell_D$, then the two pairings may belong to the same factor of the induced composition. Thus, the algorithm replaces pairing $u_k$ in $\varepsilon$ with the shortest maximal prefix-pairing $u'_k$ of $u_k$ and the longest maximal suffix-pairing $u_{k+1}$ of $v_{k+1}$, such that both $u'_k$ and $u_{k+1}$ do not overlap and have lengths at least as large as $\ell_E$. The second case is when $(t_{k+1} - t_k) - (p_{k+1} - p_k) \geq \ell_I$. In such case the two pairings might be separated by an intron. Thus, some basic biological criteria are used to compute two maximal sub-pairings $u'_k$ of $u_k$ and $u_{k+1}$ of $v_{k+1}$ that could represent a suffix and a prefix (respectively) of an exon. We replace $u_k$ with $u'_k$ and we extend the embedding with $u_{k+1}$. Whenever more than one such a pairing exists, the embedding $\varepsilon$ is extended into a set of embeddings, one for each pairing found.

Case (b). Factors $u_k$ and $v_{k+1}$ overlap on $T$ but not on $P$. This case is equivalent to the first sub-case of Case (a).

Case (c). Factors $u_k$ and $v_{k+1}$ overlap on $P$ but not on $T$. This case is similar to the entire Case (a). Notice that when the second subcase is relevant, that is $(t_{k+1} - t_k) - (p_{k+1} - p_k) \geq \ell_I$, then the splice site placement is ambiguous because a suffix of the donor exon is equal to a prefix of the acceptor exon. Also in this case, basic biological criteria are used to reduce the impact of the ambiguity.

Case (d). Factors $u_k$ and $v_{k+1}$ do not overlap neither on $P$ nor on $T$. Let $G_T$ and $G_P$ be the two substrings which separate $u_k$ and $v_{k+1}$ on $T$ and $P$, respectively. Since $G_P$ and $G_T$ do not form a pairing, they must contain a certain number of mismatches; we must evaluate if they support the hypothesis that (i) $u_k$ and $v_{k+1}$ are part of the same factor or (ii) there is an intron between
Similarly to Case (a), two different sub-cases may arise: If \( |t_{k+1} - t_k| - (p_{k+1} - p_k) | \leq \ell_D \), then \( u_k \) and \( v_{k+1} \) might belong to the same factor of the induced composition. More precisely, \( u_k \) and \( v_{k+1} \) belong to the same factor if the edit distance between \( G_T \) and \( G_P \) is below a certain threshold – in which case \( v_{k+1} \) is added to embedding \( \varepsilon \), otherwise the edge is discarded from the visit. Instead, if \( t_{k+1} - t_k - p_{k+1} + p_k \geq \ell_I \), the two pairings are separated by an intron, and we must determine the splice sites of such an intron. In this case, the algorithm computes a prefix \( G'_{T} \) and a suffix \( G''_{T} \) of \( G_T \), that minimize the edit distance between \( G_P \) and the concatenation of \( G'_T \) and \( G''_T \). Also in this case, if the resulting edit distance is larger than an acceptable threshold, the edge \((u_k, v_{k+1})\) is discarded, otherwise \( v_{k+1} \) is added to \( \varepsilon \). Notice that computing the edit distance is not too expensive, since all strings involved are no longer than \( 2\ell_D \).

The definition of embedding graph (Def [3.1]) allows the presence of directed cycles, which potentially might be troublesome. However, cycles are of no biological interest, as no actual embeddings can be associated to any cycle, therefore we simply ignore any edge ending in an already visited vertex. In fact, we claim that the embeddings, computed from a path \( P \) containing a cycle \( C \), would induce (in the following step of this stage) compositions with essentially the same set of factors of the compositions induced by the embeddings computed from the visit of the simple path \( P \setminus C \). We support our claim with a simple example on paths containing a directed cycle of length 2, that is the presence of a pair \((v_1, v_2)\) and \((v_2, v_1)\) of consecutive edges. Let \( P = \langle v_1, v_2, v_3 \rangle \) be such a path and, for simplicity, suppose that the relative position of \( v_1 \) and \( v_3 \) is the one depicted in the second sub-case of Case (d). Observe that the relative position of \( v_1 \) and \( v_2 \) must be the one of Case (a), whatever direction is considered, since a gap between the two pairings (on \( P \) or on \( T \)) would imply the absence of one of the parallel edges. Moreover, only the first sub-case of Case (a) may arise.

After the visit of path \( P \), the set \( E \) of embeddings will contain an embedding \( \varepsilon = \langle v'_1, v'_2, v'_1, v_3 \rangle \) where \( v'_1 \) is a prefix-pairing of \( v_1 \), \( v_2 \) is contained in \( v_2 \), and \( v'_1 \) is a suffix-pairing of \( v_1 \). As explained in the first sub-case of Case (a), the following step of composition reconstruction will “merge” pairings \( v'_1, v'_2, \) and \( v'_1 \) into a single factor (exon) since they are not separated by a gap large enough to represent a plausible intron. Such a factor will be approximately equal to the factor represented by pairing \( v_1 \) because \( v'_1 \) and \( v'_1 \) are, respectively, a prefix-pairing and a suffix-pairing of \( v_1 \). Instead, the visit of simple path \( P' = \langle v_1, v_3 \rangle \) computes the embedding \( \varepsilon' = \langle v_1, v_3 \rangle \). Clearly, also in this case, one of the factors computed in the following step of composition reconstruction will be (approximately) the factor represented by pairing \( v_1 \), concluding the example.

The visit performed in the first step of algorithm ComputeCompositions guarantees that each possible representative embedding is analyzed. However, the biological criteria that we employ allow to consider only pairings belonging to biologically meaningful embeddings. Since the visit computes pairwise-distinct representative embeddings and every case presented above requires \( O(1) \) time, the overall computational complexity of the visit is clearly bounded by \( O(\sum_{\varepsilon \in E} |\varepsilon|) \), that is the total size of the representative embeddings that have
been computed during the visit.

**Composition reconstruction.** The set $E$ of representative embeddings computed by the visit of the embedding graph directly leads to a set $C$ of compositions. In fact, the visit guarantees that two consecutive pairings of a representative embedding are either separated by a “small” gap due to errors or by a “large” gap representing an intron of the spliced alignments. Hence, the algorithm simply merges into a factor a sequence of consecutive pairings $v_k = (p_k, t_k, l_k)$ and $v_{k+1} = (p_{k+1}, t_{k+1}, l_{k+1})$ separated by “small” gaps, that is $|t_{k+1} - t_k - p_{k+1} + p_k| \leq \ell_D$. Finally, the composition is retained if the edit distance between each EST factor and the corresponding genomic factor is not greater than a fixed acceptable threshold.

### 3.3 Building a Gene Structure

In the third stage of our pipeline, we compute a maximum-parsimony consensus gene-structure starting from the compositions of a cluster of transcript sequences against a common genomic sequence. Let $T$ be the genomic sequence of a given gene locus and let $S = \{P_1, \ldots, P_k\}$ be the cluster of transcript sequences that map to the gene locus. The first two stages of our pipeline have considered separately each transcript sequence $P_i$ and, for each of them, a set $C(P_i)$ of biologically meaningful compositions has been computed. The main task is to extract a composition for each transcript that explains the putative gene structure. The redundancy of information, due to compositions of different transcripts in the cluster, is an important ingredient to discover a single composition of each transcript that agrees with the gene structure. To achieve this goal we apply the Minimum Factorization Agreement (MFA) problem [3].

Let us recall the definition of the MFA problem. Let $S$ be a set of sequences over a finite alphabet $\Sigma$ of symbols, and let $F = \langle f_1, f_2, \ldots, f_{|F|} \rangle$ be a finite ordered set of sequences over alphabet $\Sigma$, called factors. Given a sequence $s \in S$, a factor-composition (f-composition in short) of $s$ consists of the sequence $\langle f_{i_1}, f_{i_2}, \ldots, f_{i_n} \rangle$ such that $s = f_{i_1}, f_{i_2}, \ldots, f_{i_n}$ and $i_j < i_{j+1}$ for $1 \leq j \leq n - 1$. While the notion of f-composition depends on the set of factors, such set of factors is usually clear from the context and is therefore omitted in the definition. Please notice that a sequence $s$ can admit different f-compositions: thus let $F(s)$ be the set of compositions of $s$. Moreover, by extension, we will denote by $F(S) = \cup_{s \in S} F(s)$ the set of f-compositions of a set $S$ of sequences.

Given a f-composition $f = \langle f_{i_1}, f_{i_2}, \ldots, f_{i_n} \rangle$, the set $\{f_{i_1}, f_{i_2}, \ldots, f_{i_n}\}$ is called the factor set of $f$ and is denoted as $F(f)$. Given a subset $F' \subseteq F$ of factors and the set $F(S)$, then $F'$ is a factorization agreement set for $F(S)$ if and only if for each sequence $s \in S$, there exists a f-composition $f$ in $F(s)$ such that its factor set is a subset of $F'$, i.e. $F(f) \subseteq F'$.

The Minimum Factorization Agreement problem, given a set $F$ of factors and a set $S$ of sequences, asks for a minimum cardinality subset $F' \subseteq F$ such that $F'$ is a factorization agreement set for $F(S)$. Informally, the solution to the MFA problem is a smallest set of factors that is able to explain a f-composition
for each input sequence. In our setting \( S \) is the cluster of transcript sequences and \( F \) is the set of all exons (factors) used to produce the compositions of sequences in \( S \), i.e. \( F(S) \) consists of all the compositions of \( S \). When solving the MFA problem on these input data, the solution \( F' \) provides a minimum set of factors that can explain all transcript sequences and a single composition of each transcript can be obtained from set \( F' \).

However, in order to apply the MFA problem to our data we need to define a binary relation between factors (exons). Indeed, the first and the last factors of different transcript sequences could be fragments of the same exon. Moreover, since internal factors of transcript compositions are computed without applying refined biological criteria for the location of splice junctions, even internal factors of different transcript compositions could be associated to the same exon whenever they differs by few bases. For this reason we define a binary relation between factors of compositions. More precisely, we say that two factors \( f \) and \( f' \) are \( \sim \)-related if the two factors share a common overlapping regions of length at least \( N \), for \( N \) a fixed bound. Factors that are \( \sim \)-related are grouped into a region: each composition \( c \) of a transcript sequence \( s \) is then replaced by a composition into regions corresponding to each factor (recall that regions are disjoint). Similarly the set \( F \) consists of regions and thus the MFA problem applied to the new set \( F(S) \) produces a minimum set \( F' \) of regions that could explain all input compositions.

By applying the algorithm proposed by [3] we are able to filter efficiently a set of spliced alignments agreeing to the same gene structure that are further refined by the next step of intron reduction.

3.4 Intron Reduction

Although the intron boundaries of EST spliced compositions are computed by finding the best transcript-genome alignment over the splice site regions and the most frequent intron pattern (i.e. the first and the last two nucleotides of an intron) according to [6], the set of predicted introns may still contain false positives very close to true predictions. This can be explained by the example in Figure 2. Let us suppose that there exists an EST \( s_1 \) (see Figure 2(a)) producing a canonical intron \( i \) – i.e. satisfying the so-called \( GT - AG \) rule [6]
– and that \( s_1 \) perfectly matches to the genomic sequence over the intron splice sites. Moreover, let us suppose that there exists another EST \( s_2 \) (see Figure 2(b)) with a deletion of a base 'T' with respect to \( s_1 \) (highlighted in boldface in Figure 2(a)). Because of our spliced alignment algorithm, \( s_2 \) produces a non-canonical intron \( i' \) (indeed its pattern is \( TA - GT \)), which differs from \( i \) by only a few bases. We can reasonably assume that \( i' \) is a false positive and not a reliable prediction. Hence, the spliced composition of \( s_2 \) can be corrected into the spliced alignment supporting the intron \( i \) (see Figure 2(c)), by introducing just one insertion error into the genomic sequence. Thus, a procedure for comparing the intron set computed by the EST spliced compositions in order to correct and reduce the set of false positives (i.e. over predicted introns) is necessary.

In the following, let the pair \((i, s)\) denotes a genomic intron (eventually specified by a pair of genomic coordinates) and a spliced composition of an EST \( s \) supporting the intron \( i \), i.e. having two consecutive factors \( f_i, f_{i+1} \) inducing intron \( i \) when aligned to the genome. Then, given an error bound \( b \), we say that \((i, s)\) is \( b \)-reducible to \((i', s)\) iff there exists a boundary shift of factors \( f_i \) and \( f_{i+1} \) of a new spliced composition of \( s \) inducing intron \( i' \) with at most additional \( b \) errors w.r.t. the previous alignment of the two factors against the genome.

Since RefSeq transcripts are usually full-length and error-free, and \( GT - AG \), \( GC - AG \) and \( AT - AC \) rules are the most frequent [6] and are associated to U12/U2 introns [18], we assume that only introns, that do not follow one of the U12/U2 rules and are not supported by a RefSeq transcript should be reduced.

The input of our intron-reduction procedure is a set \( X \) of pairs \((i, s)\) computed by the previous steps. Then, \( R \) is the set of pairs in \( X \) such that \( s \) is a RefSeq, \( C_1, C_2, C_3 \) and \( N \) are the set of pairs in \( X \setminus R \) following the \( GT - AG \), \( GC - AG \), \( AT - AC \) and a non-U12/U2 rule respectively. Our procedure basically tries to reduce elements in \( N \) to some intron in \( R \) (i.e. the most reliable class composed by RefSeq-supported introns) and if this is not possible tries to reduce to some element in the first set of the sequence \( C_1, C_2, C_3 \) that allows the reduction.

### 4 Implementation

We implemented the pipeline as a set of C programs in the software package PIntron. The input is a genomic sequence \( G \) and a set \( T \) of EST and mRNA sequences, referred in the following with the more general term of transcripts. Our method computes a set \( C \) of exon compositions of the sequences in \( S \) with respect to the genomic sequence, such that there exists one exon composition per input transcript. In fact, for each transcript, our software retains only those compositions that can possibly provide information about the exon-intron structure. Our implementation is still at a preliminary stage; in fact only a few basic biological criteria have been introduced, such as constraints on the intron lengths as well as on the size of removable transcript prefixes and suffixes. The main goal of our study – and of our current implementation – is to assess the efficiency of the pipeline while maintaining a reasonable quality of the results produced. Together with the exon compositions, PIntron outputs the positions,
Figure 3: Distribution of ENCODE introns (left) and splicing sites (right) confirmed by PIntron. For each region we have represented the mean of sensitivity and specificity values, together with the interval ranging from the mean minus the standard deviation and the mean plus the standard deviation.

on the genomic sequence, of the donor and the acceptor splice sites of the introns, as well as several additional informations included the intron type (U12, U2 or unclassified) [18].

5 Discussion

The results of the experimental analysis are very positive, especially the running times, as the analysis of several genes has required only a few hours, even using modest computational resources. After a first experiment, where we have analyzed a set of 112 fairly typical genes from 13 ENCODE regions with lengths ranging from 0.5Mb to 1.7Mb encompassing 98,064 transcripts (with total length 63Mb), we have decided to investigate the scalability of our implementation by analyzing some hand-picked genes exhibiting a large genomic sequence or a large set of transcripts. Comprehensive data regarding the experiments are reported in the supplementary material.

In the first experiment, for each gene the associated human Unigene cluster (including also RefSeq mRNAs) has been processed by our spliced alignment algorithm, together with the whole ENCODE region. We tested our software on an off-the-shelf PC with a total running time of 1h 17sec (on average 41 seconds/gene).

On the whole set of the 112 input genes, 1787 out of 1957 benchmark introns are also in the test set with an average support of 214.45 transcripts per intron (not including outlier genes RPL10 and EEF1A1 that have 6,414 and 49,936 transcripts respectively). We have further investigated the 170 ENCODE introns that have not been predicted by our method, searching in the ENSEMBL Genome Browser the supporting transcripts of each unpredicted intron. The investigation pointed out that 95 introns (55.88% over the total of missing data) have no evidence in the ENSEMBL database, hence they are probably hand-
annotated introns. Of the remaining 75 unpredicted introns, 41 (24.12%) have no supporting transcripts in the Unigene set fed to our implementation. 18 unpredicted introns (10.59%) have some supporting transcripts, but with only low-quality alignments between each transcript and the unpredicted intron. For most such transcripts our method computes a high-quality alignment supporting another ENCODE intron that is very close to the unpredicted one. Finally, for 16 (9.41%) unpredicted introns our method is unable to align the transcripts supporting such introns (in one case we found errors in the Unigene DB). The overall sensitivity of our method is 0.9813, computed after discarding from the benchmark set the 136 ENCODE introns that have no evidence in ENSEMBL DB or have no supporting transcripts in the Unigene cluster given in input to PIntron. The benchmark set contains no intron for F8A1, H2AFB1 and IER5L. Our method confirms ENCODE for the first two genes, while it predicts 3 introns for IER5L. Notice that all genes (except OPN1MW) have sensitivity at least 0.8 and almost 75% have sensitivity 1.

Our experiment has predicted 1042 introns that are not in the benchmark. The overall specificity of our method is 0.6317 and 10 genes have specificity equal to 1. Moreover, the average number of supporting transcripts for each new intron is 2.42. 268 new predictions have one of the two splice sites in the benchmark set B, while 92 new introns have both splice sites in B (but they belong to different ENCODE introns). Sensitivity and specificity for this analysis are represented in Figures 3 and 4.

We have also studied if the introns predicted by PIntron are close to those predicted by ENCODE, relaxing the definition of introns common to the benchmark and test sets by allowing splice sites that differ for up to 8 positions. We omit the results as no significant improvement has resulted.

Since our pipeline seems to overpredict with respect to ENCODE, we have started a detailed analysis to understand the causes of such overpredictions. Almost all introns predicted by PIntron and ENCODE (1778 out of 1787) are canonical, classified as U12/U2 and contain a Branch Point Sequence (BPS).
Since we have not completed our analysis yet, we are currently unable to determine if being canonical, classified or containing a BPS should be a requirement for a putative intron to be retained in the results output by PIntron. For example, introducing the criterion that only canonical and classified are output, the number of new predictions decreases from 1042 to 565: in such case the sensitivity becomes 0.9764 and the specificity becomes 0.7598.

Other comparisons among gene-structure prediction tools in literature [4] are performed over different datasets, therefore that analysis and ours are not completely comparable. Anyway, for illustrative purposes we report that two commonly used software packages for predicting the exon-intron structure, namely ECgene [16] and ASPic [8], have sensitivity/specificity that is respectively 0.92/0.63 and 0.88/0.77 [4]: not as good as those obtained by our pipeline. Another parameter that is surely interesting is the number of EST supporting a exon/intron.

The time performance of PIntron over the experimented ENCODE genes is detailed in the supplementary material. In this section we will show the results only for three genes that are critical because of the size of the input transcripts or because of the length of the input genome (i.e. the corresponding ENCODE region). We will report only the time for computing spliced compositions, since it is the most time-consuming part of our pipeline. More precisely, gene TIMP3 has taken 48 seconds for computing spliced compositions of 1,700 transcripts against the ENCODE region ENm004 that is 1,700,000 bp long. Gene RPL10 (in region ENm006) has taken 141 sec for aligning 6,414 transcripts against a genome of 1,338,447bp, and finally the time, for computing the spliced compositions of the 49,936 transcripts of gene EEF1A1 against the 500,000bp of region ENr223, has been 416 sec.

To determine the scalability of our approach, we have chosen 26 genes, of which 11 are at least 1Mb long (on average about 848Kb), and 5 have more than 15,000 transcripts (on average more than 5000 transcripts). We tested our software on a commercial workstation equipped with 12GB of RAM with a total running time of 20 min (average 47 seconds/gene). Notice that processing TTN gene has taken 545 seconds (while other tools, such as the aforementioned ECgene, do not provide predictions for this gene). The likely reason is that the input set contains transcripts (ESTs and mRNAs) that are more than 80Kb long, and the spliced alignment computation time is very high for long EST sequences since their quality is lower than the quality of mRNA sequences.

An analysis on the running times from both experiments has not shown any significant correlation between the length of the genes and the running times, hence confirming our conjecture that the behaviour of our algorithm depends on some properties of the embedding graph, and not on the size of the instance. In particular, the structure of the embedding graph is strictly related to the quality of the transcripts and to the presence in the gene of repetitions, highly duplicated regions or other elements that could influence the size of the graph. The results have confirmed our beliefs, since the average running time of the second experiment (47 seconds/gene), albeit on a faster PC, is not too far from the running times on the much smaller genes of the first experiment, where the average value is 41 seconds/gene. The experimental results for all investigated
genes are available at \url{http://www.algolab.eu/PIntron}

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