Alignment of protein-coding sequences with frameshift extension penalties

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Abstract. We introduce an algorithm for the alignment of protein-coding sequences accounting for frameshifts. The main specificity of this algorithm as compared to previously published protein-coding sequence alignment methods is the introduction of a penalty cost for frameshift extensions. Previous algorithms have only used constant frameshift penalties. This is similar to the use of scoring schemes with affine gap penalties in classical sequence alignment algorithms. However, the overall penalty of a frameshift portion in an alignment cannot be formulated as an affine function, because it should also incorporate varying codon substitution scores. The second specificity of the algorithm is its search space being the set of all possible alignments between two coding sequences, under the classical definition of an alignment between two DNA sequences. Previous algorithms have introduced constraints on the length of the alignments, and additional symbols for the representation of frameshift openings in an alignment. The algorithm has the same asymptotic space and time complexity as the classical Needleman-Wunsch algorithm.

Keywords: Protein-coding sequences, Pairwise alignment, Frameshifts, Dynamic programming

1 Introduction and motivation

Comparative genomics is currently facing a huge challenge with the revelation of a growing number of genes having multiple alternative coding sequences in several species [4,10]. The various coding sequences arising from a same gene or homologous genes differ not only by mutations in the nucleotide sequences, but also by alternative start codons and alternative splicing of exons. All these mechanisms often induce translation frameshifts that lead to different translations of a same portion of gene in distinct coding sequences [8]. This new enlightenment on the complexity of gene architecture evolution calls for novel algorithms for the comparison of coding sequences capable to account for the presence of translation frameshifts between coding sequences.

The problem of aligning two coding sequences is an optimization problem that consists in finding an optimal score alignment in a set of alignments between the two sequences. A coding sequence is a DNA sequence composed of a succession of words of length 3 called codons. An alignment between two DNA
sequences $A$ and $B$ is a pair of sequences $A'$ and $B'$ of same length $L$ on the alphabet of nucleotides augmented with the gap symbol '-', such that $A'$ and $B'$ do not contain a gap symbol '-' at a same position, and $A$ and $B$ can be derived from $A'$ and $B'$ by removing all the gap symbols. The length $L$ of $A'$ and $B'$ is called the length of the alignment. A translation frameshift in an alignment between two coding sequences is caused by i) the deletion of one or two nucleotides of a codon (for example, a codon $\text{ACC}$ aligned with $A--$), or ii) the insertion of nucleotides between two nucleotides of a codon (for example, a codon $A--\text{CC}$ aligned with $\text{AGACC}$). The computation of an optimal alignment between two coding sequences should account for both the translation of the coding sequences into protein sequences, and the presence of translation frameshifts between the two coding sequences.

A classical approach for comparing two coding sequences consists in a three-step method, where coding sequences are first translated into protein sequences, next protein sequences are aligned, and finally the protein alignment is back-translated to a coding sequence alignment. This approach is used in most tools for multiple alignment of coding sequences [1,14,3,6]. However, it is not able to account for the presence of frameshifts between coding sequences.

The problem of aligning two coding sequences of length $n$ and $m$ while accounting for both the corresponding protein sequences and the presence of frameshifts was first addressed by Hein et al. [5,9]. They proposed a DNA/protein model such that the score of an alignment between two coding sequences is a combination of its score at the DNA level and its score at the protein level. Under this model, a $O(n^2m^2)$ algorithm [5] and then a $O(nm)$ algorithm [9] were proposed to compute an optimal score alignment. The search space of the algorithms are the set of alignments that can be each uniquely decomposed into a succession of sub-alignments of eleven (11) types. The eleven types of sub-alignment are defined such that the length of each of them is a multiple of 3. Thus, the total length of any alignment in the search space is always a multiple of 3, and the score of an alignment is the sum of the scores of its sub-alignments.

Arvestad [2] proposed another $O(nm)$ protein-coding alignment algorithm based on the concept of generalized substitutions introduced in [12]. In this algorithm, an alignment between two coding sequences $A$ and $B$ is a pair of sequences on the alphabet of nucleotides augmented with the gap symbol '-' and the frameshift symbol '!'. The search space of the algorithm is the set of alignments that are each composed of a succession of sub-alignments of length 3 such that each sub-alignment is an alignment between two codon fragments of $A$ and $B$. A codon fragment of a coding sequence $S$ is defined as a word of length 0 to 5 in $S$. If a codon fragment has a length of 4 (resp. 5), then one or two nucleotides in the codon fragment are dropped in order to fit in a sub-alignment of length 3. Such dropped nucleotides are simply ignored in the definition of the score of a length-3 sub-alignment. If a codon fragment has a length of 1 or 2, then two or one frameshift opening symbols '!' are added in the codon in order to fit in a sub-alignment of length 3. The score of an alignment is then defined as the sum of the scores of its length-3 sub-alignments.
More recently, Ranwez et al. [11] proposed a simplification of the model of Arvestad [2] where a *codon fragment* of a coding sequence $S$ is defined as a word of length 0 to 3 in $S$. Thus, no supplemental combinatorics are required in order to consider all the possibilities of dropping one or two nucleotides from a codon fragment of length 4 or 5. The algorithm has a complexity in $O(n.m)$.

This method was extended in the context of multiple protein-coding sequence alignment [11]. The above three methods [2,9,11] compare two coding sequences while accounting for the presence of translation frameshift openings between the two sequences. A frameshift in an alignment is penalized by adding a constant frameshift cost, which only penalizes the initiation of a frameshift, not accounting for the extension of this frameshift in the alignment.

For example, we consider the following three coding sequences: Seq1, Seq2, and Seq3. Seq1 has a length of 45. Seq2 (resp. Seq3) has a length of 60 and is obtained from Seq1 by deleting the nucleotide 'C' at position 30 (nucleotide 'G' at position 15) and adding 16 nucleotides at the end.

Seq1: ATGACCAATCCCAAGCAGCCCTGGCATAAAGTGGGGAAACGATTGA
     M T E S K Q P W H K W G N D *
Seq2: ATGACCAATCCCAAGCAGCCCTGGCATAAATGGGGGAACGATGAAGTAGGAACGATTAA
     M T E S K Q P W H N G C T I E V G T I *
Seq3: ATGACCAATCCCAAGCAGCCCTGGCATAAAGTGGGGAAACGATGAAGTAGGAACGATTAA
     M T E S N S P G I S G C T I E V G T I *

When looking at the translations of Seq1 and Seq2, it is easily observable that Seq2 is more similar to Seq1, than Seq3 is similar to Seq1. However, the pairwise alignment algorithms accounting for frameshifts [2,9,11] would return the same score for the two following optimal alignments of Seq1 and Seq2, and Seq1 and Seq3, penalizing only the initiation of a frameshift in both cases (positions colored in red in the alignments).

**Optimal alignment between Seq1 and Seq2:**

```
M T E S K Q P W H K W G N D * - - - - - -
ATGACCAATCCCAAGCAGCCCTGGCATAAATGGGGGAACGATTGA------------------
ATGACCAATCCCAAGCAGCCCTGGCATAA-TGGGGGAACGATTGAAGTAGGAACGATTAA--
M T E S K Q P W H ! W G N D * S R N D L !
```

**Optimal alignment between Seq1 and Seq3:**

```
M T E S K Q P W H K W G N D * - - - - - -
ATGACCAATCCCAAGCAGCCCTGGCATAAATGGGGGAACGATTGA------------------
ATGACCAATCCCAAGCAGCCCTGGCATAAAGTGGGGAAACGATGAAGTAGGAACGATTAA--
M T E S ! Q P W H K W G N D * S R N D L !
```

We describe a pairwise alignment algorithm that uses a scoring scheme penalizing both the initiation and the extensions of frameshifts (positions colored in blue in the alignments). In Section 2, some preliminary definitions of alignments and the description of the problem are presented. In Section 3, the new algorithm for computing an optimal score alignment is described.
2 Preliminaries : Alignment of protein-coding sequences

In this section, we formally describe coding sequences and the pairwise alignment problem that is solved in Section 3.

Definition 1 (Coding sequence). A coding sequence is DNA sequence on the alphabet of nucleotides $\Sigma_N = \{a,c,g,t\}$ whose length $n$ is a multiple of 3. A coding sequence is composed of a succession of $\frac{n}{3}$ codons that are the words of length 3 in the sequence ending at positions $3i, 1 \leq i \leq \frac{n}{3}$. The translation of the coding sequence is a protein sequence of length $n$ on the alphabet $\Sigma_A$ of amino acids (aa) such that each codon of the coding sequence is translated into an amino acid in the protein sequence.

In this work, the definition of an alignment between two coding sequences is exactly the same as the classical definition of an alignment between two DNA sequences used by the Needleman-Wunsch algorithm for the comparison of two sequences [7].

Definition 2 (alignment between DNA sequences). An alignment between two DNA sequences $A$ and $B$ is a pair $(A', B')$ where $A'$ and $B'$ are two sequences of same length $L$ derived by inserting gap symbols $'-'$ in $A$ and $B$, such that $\forall i, 1 \leq i \leq L, A'[i] \neq '-'$ or $B'[i] \neq '-$. Each position $i, 1 \leq i \leq L$, in the alignment is called a column of the alignment.

Given a sequence $S$ of length $L$ on the alphabet $\Sigma = \{a,c,g,t,-\}$, $S[k..l], 1 \leq k \leq L$, denotes the subsequence of $S$ going from position $k$ to position $l$. $|S[k..l]|$ denotes the number of letters in $S[k..l]$ that are different from the gap symbol $'-'$. For example, $|AC--G| = 3$.

Given an alignment $(A', B')$ between two coding sequences $A$ and $B$, a codon of $A$ or $B$ is grouped in the alignment if its three nucleotides appear in three consecutive columns of the alignment. For example, a codon ACC that appears in the alignment as ACC is grouped, while it is not grouped if it appears as A-CC.

In the following, we give our definition of the score of an alignment between two coding sequences $A$ and $B$. It is based on a partition of the codons of $A$ and $B$ into four sets (types):

The set of Matching codons (M) contains the codons that are grouped in the alignment, and aligned exactly with a codon of the other sequence.

The set of Unmatching codons (U) contains the codons that are grouped in the alignment, and aligned with three consecutive nucleotides of the other sequence that do not form a codon.

The set of Deleted/Inserted codons (InDel) contains the codons that are grouped in the alignment, and aligned with a succession of 3 gaps.

All other codons are frameshift codons. Following the definitions and notations for frameshifts used in [11], the set of frameshift codons can be divided into two sets. The set of frameshift codons caused by deletions (FS$^-$) contains the codons that are grouped in the alignment, and are aligned with only one or two nucleotides in the other sequence and some gap symbols. The set
of frameshift codons caused by insertions \((\text{FS}^+)\) contains all the codons that are not grouped in the alignment.

The set of Matching nucleotides in frameshift codons \((\text{MFS})\) contains all the nucleotides belonging to a frameshift codon, and aligned with a nucleotide of the other sequence.

The substitutions of matching \((\text{M})\) and unmatching \((\text{U})\) codons are scored using an amino acid scoring function \(s_{\text{aa}}\), and a fixed frameshift extension cost denoted by \(\text{fs-extension cost}\) is added for each unmatching codon \((\text{U})\). The insertions/deletions of codons \((\text{Indel})\) are scored by adding a fixed gap cost denoted by \(\text{gap cost}\) for each inserted/deleted codon \((\text{Indel})\). The alignment of frameshift codon nucleotides \((\text{MFS})\) are scored independently from each other, using a nucleotide scoring function \(s_{\text{aa}}\). The insertions or deletions of nucleotides from frameshift codons are responsible for the initiation of frameshifting. They are then scored by adding a fixed frameshift opening cost denoted by \(\text{fs-open cost}\) for each frameshift codon.

In the following definition of the score of an alignment, the matching \((\text{M})\), unmatching \((\text{U})\), and deleted/inserted \((\text{InDel})\) codons of \(A\) and \(B\) are simply identified by the position (column) of their last nucleotide in the alignment. The matching nucleotides in frameshift codons \((\text{MFS})\) are also identified by their positions in the alignment.

**Definition 3 (Score of an alignment).** Let \((A', B')\) be an alignment of length \(L\) between two coding sequences \(A\) and \(B\).

\[
M_{A \to B} = \{ (k, k) \leq L \mid 3 \{ (i, j) \text{ s.t. } A'[k-2 \ldots k] = A[i-2 \ldots 3i] \text{ and } B'[k-2 \ldots k] = B[3j-2 \ldots 3j] \}\}
\]
\[
U_{A \to B} = \{ (k, k) \leq L \mid k \notin M_{A \to B} \text{ and } 3 \{ i \text{ s.t. } A'[k-2 \ldots k] = A[3i-2 \ldots 3i] \text{ and } |B'[k-2 \ldots k]| = 3 \}\}
\]
\[
\text{Indel}_{A \to B} = \{ (k, k) \leq L \mid 3 \{ i \text{ s.t. } A'[k-2 \ldots k] = A[3i-2 \ldots 3i] \text{ and } |B'[k-2 \ldots k]| = 0 \}\}
\]
\[
\text{MFS}_{A \to B} = \{ (k, k) \leq L \mid \exists \{ (i, j) \text{ s.t. } A'[k-2 \ldots k] = A[i-2 \ldots 3i] \text{ and } |B'[k-2 \ldots k]| = 3 \}\}
\]
\[
M_{B \to A} = \{ (k, k) \leq L \mid 3 \{ (j, i) \text{ s.t. } B'[k-2 \ldots k] = B[3j-2 \ldots 3j] \text{ and } A'[k-2 \ldots k] = A[3i-2 \ldots 3i] \}\}
\]
\[
U_{B \to A} = \{ (k, k) \leq L \mid k \notin M_{B \to A} \text{ and } 3 \{ j \text{ s.t. } B'[k-2 \ldots k] = A[3j-2 \ldots 3j] \text{ and } |A'[k-2 \ldots k]| = 3 \}\}
\]
\[
\text{Indel}_{B \to A} = \{ (k, k) \leq L \mid 3 \{ j \text{ s.t. } B'[k-2 \ldots k] = B[3j-2 \ldots 3j] \text{ and } |A'[k-2 \ldots k]| = 0 \}\}
\]
\[
\text{MFS}_{B \to A} = \{ (k, k) \leq L \mid \exists \{ (j, i) \text{ s.t. } B'[k-2 \ldots k] = B[j] \text{ and } A'[k] = A[i] \}\}
\]

The score of the alignment \((A', B')\) is defined by:

\[
\text{score}(A') = \sum_{k \in M_{A \to B}} s_{\text{aa}}(A'[k-2 \ldots k], B'[k-2 \ldots k]) + \sum_{k \in U_{A \to B}} \left( s_{\text{aa}}(A'[k-2 \ldots k], B'[k-2 \ldots k]) + \text{fs-extension cost} \right) + \left| M_{A \to B} \right| \* \text{gap cost} + \left( \frac{|A|}{3} - |M_{A \to B}| - |U_{A \to B}| - |\text{Indel}_{A \to B}| \right) \* \text{fs-open cost} + \sum_{k \in \text{MFS}_{A \to B}} s_{\text{aa}}(A'[k], B'[k]) \]

\[ \text{score}(B') = \sum_{k \in M_{B-A}} s_{aa}(B'[k-2 .. k], A'[k-2 .. k]) + \sum_{k \in U_{B-A}} (s_{aa}(B'[k-2 .. k], A'[k-2 .. k]) + \text{fs\_extension\_cost}) + (|\text{Indel}_{B-A}| + \text{gap\_cost}) + (|B| - |M_{B-A}| - |U_{B-A}| - |\text{Indel}_{B-A}|) + \text{fs\_open\_cost} + \sum_{k \in \text{MFS}_{B-A}} s_{aa}(B'[k], A'[k])/2 \]

\[ \text{score}(A', B') = \text{score}(A') + \text{score}(B') \]

For example, consider the two following sequences, \( A \) containing 13 codons and \( B \) containing 14 codons, and an alignment of length 48 between them.

\( A: \text{ATGACCGAATCCAAGCAGCCCTGGCCAGATCAACGTTGA} \)
\( B: \text{ATGGAGTCGAAGATCAGCTGGCAGGCCATTGGCAATGACTGA} \)

An alignment \((A',B')\) of length 48 between \( A \) and \( B \):

\( \text{score}(A', B') = 10 \)

**Algorithm**

In this section, we describe a \( O(nm) \) time and space complexity algorithm that solves the problem of finding a maximum score alignment between two coding sequences \( A \) and \( B \) of lengths \( n \) and \( m \). Similarly to other sequence comparison methods [7, 13], we use dynamic programming tables of size \( n \times m + 1 \) that are indexed by the pairs of prefixes of the two coding sequences. The table \( D \) stores the maximum scores of the alignments between prefixes of \( A \) and \( B \). The table \( D_F \) is used to account for potential cases of frameshift extensions that are counted subsequently.

**Definition 4 (Dynamic programming tables).** Given two coding sequences \( A \) and \( B \) as input, the algorithm uses two dynamic programming tables \( D \) and \( D_F \) of size \( n \times m + 1 \). The cell \( D(i, j) \) contains the maximum score of an alignment between the prefixes \( A[1 .. i] \) and \( B[1 .. j] \). The table \( D_F \) is filled only for values of \( i \) and \( j \) such that \( i/(\text{mod} \ 3) = 0 \) or \( j/(\text{mod} \ 3) = 0 \). If \( i/(\text{mod} \ 3) \neq 0 \) (resp. \( j/(\text{mod} \ 3) \neq 0 \)), the cell \( D_F(i, j) \) contains the score of an alignment between
the prefixes $A[1..i+\alpha]$ and $B[1..j+\alpha]$ where $\alpha = (3 - i)(\text{mod} \ 3)$ (resp. $\alpha = (3 - j)(\text{mod} \ 3)$). The table $D_F$ is filled as follows:

- If $i(\text{mod} \ 3) = 0$ and $j(\text{mod} \ 3) = 0$, $D_F(i, j) = D(i, j)$.
- If $i(\text{mod} \ 3) = 0$ and $j(\text{mod} \ 3) = 1$, or $i(\text{mod} \ 3) = 1$ and $j(\text{mod} \ 3) = 0$, $D_F(i, j)$ contains the maximum score of an alignment between $A[1..i+1]$ and $B[1..j+1]$ such that $A[i+1]$ and $B[j+1]$ are aligned together, and half of the score for aligning $A[i+1]$ with $B[i+1]$ is subtracted.
- If $i(\text{mod} \ 3) = 0$ and $j(\text{mod} \ 3) = 2$, or $i(\text{mod} \ 3) = 2$ and $j(\text{mod} \ 3) = 0$, $D_F(i, j)$ contains the maximum score of an alignment between $A[1..i+1]$ and $B[1..j+1]$ such that $A[i+1], B[j+1]$ and $A[i+2], B[j+2]$ are aligned together, and half of the scores of aligning $A[i+2], B[i+2]$, and $A[i+1], B[i+1]$ is subtracted.

Lemma 1 (Filling up table $D$).

1. If $(i(\text{mod} \ 3) = 0$ and $j(\text{mod} \ 3) = 0$)

$$D(i, j) = \max \begin{cases} 
1. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + D(i-3, j-3) \\
2. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-1], B[j]) + D(i-2, j-2) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
3. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-2], B[j-1]) + D(i-3, j-2) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
4. \ s_{\text{un}}(A[i], B[j]) + D(i-3, j-1) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
5. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-1], B[j-1]) + D(i-2, j-3) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
6. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-1], B[j-2]) + D(i-2, j-3) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
7. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-3) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
8. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
9. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-1], B[j-2]) + D(i-2, j) + \text{fs}_{\text{open}} \text{cost} \\
10. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
11. \ s_{\text{un}}(A[i], B[j]) + D(i-2, j) + \text{fs}_{\text{open}} \text{cost} \\
12. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) \times \text{fs}_{\text{open}} \text{cost} \\
13. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j) \times \text{fs}_{\text{open}} \text{cost} \\
14. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-2], B[j-2]) + D(i-3, j-1) + \text{fs}_{\text{open}} \text{cost} \\
15. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-3) + 2 \times \text{fs}_{\text{open}} \text{cost} \\
16. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-3) + \text{fs}_{\text{open}} \text{cost} \\
17. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j) + \text{fs}_{\text{open}} \text{cost} \\
18. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j) + \text{fs}_{\text{open}} \text{cost} \\
\end{cases}$$

2. If $(i(\text{mod} \ 3) = 0$ and $j(\text{mod} \ 3) \neq 0$)

$$D(i, j) = \max \begin{cases} 
1. \ s_{\text{un}}(A[i-2], B[j-2]) + D(i-3, j-3) + \text{fs}_{\text{extension}} \text{cost} \\
2. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-1], B[j]) + D(i-3, j-2) + \text{fs}_{\text{open}} \text{cost} \\
3. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-2], B[j]) + D(i-3, j-2) + \text{fs}_{\text{open}} \text{cost} \\
4. \ s_{\text{un}}(A[i], B[j]) + D(i-3, j-1) + \text{fs}_{\text{open}} \text{cost} \\
5. \ s_{\text{un}}(A[i], B[j]) + s_{\text{un}}(A[i-1], B[j-1]) + D(i-2, j-3) + \text{fs}_{\text{open}} \text{cost} \\
6. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + \text{fs}_{\text{open}} \text{cost} \\
7. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + \text{fs}_{\text{open}} \text{cost} \\
8. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + \text{fs}_{\text{open}} \text{cost} \\
9. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + \text{fs}_{\text{open}} \text{cost} \\
10. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + \text{fs}_{\text{open}} \text{cost} \\
11. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j-1) + \text{fs}_{\text{open}} \text{cost} \\
\end{cases}$$

3. If $(i(\text{mod} \ 3) \neq 0$ and $j(\text{mod} \ 3) = 0$, the equation is symmetric to the previous case.

4. If $(i(\text{mod} \ 3) \neq 0$ and $j(\text{mod} \ 3) \neq 0$)

$$D(i, j) = \max \begin{cases} 
1. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j) \\
2. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j) \\
3. \ s_{\text{un}}(A[i], B[j]) + D(i-1, j) \\
\end{cases}$$
Case 1. \(i \mod 3 = 0\) and \(j \mod 3 = 0\)

|   | (a) i.                | (a) ii. A.       | (a) ii. B.       |
|---|-----------------------|------------------|------------------|
| 1 | \( \text{x} \times \times \) | \( \text{x} \times \) | \( \text{x} \times \) |
| 2 | \( \text{x} \times \times \) | \( \text{x} \times \) | \( \text{x} \times \) |
| 3 | \( \text{x} \times \times \) | \( \text{x} \times \) | \( \text{x} \times \) |
| 4 | \( \text{x} \times \times \) | \( \text{x} \times \) | \( \text{x} \times \) |

|   | (a) iii.              |
|---|-----------------------|
| 5 | \( \text{x} \times \) |
| 6 | \( \text{x} \times \) | \( \text{x} \times \) |
| 7 | \( \text{x} \times \times \) | \( \text{x} \times \) |

|   | (a) iv.               |
|---|-----------------------|
| 8 | \( \text{x} \times \) |

|   | (b) i. A.             | (b) i. B.       |
|---|-----------------------|------------------|
| 9 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 10| \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 11| \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 12| \( \text{x} \times \times \) | \( \text{x} \times \times \) |

|   | (b) i. C.             |
|---|-----------------------|
| 13| \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 14| \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 15| \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 16| \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 17| \( \text{x} \times \times \) | \( \text{x} \times \times \) |

|   | (c)                   |
|---|-----------------------|
| 18| \( \text{x} \times \times \) | \( \text{x} \times \times \) |

Case 2. \(i \mod 3 = 0\) and \(j \mod 3 \neq 0\)

|   | (a) i. A.             | (a) i. B.       |
|---|-----------------------|------------------|
| 1 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 2 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 3 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 4 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |

|   | (a) ii.               |
|---|-----------------------|
| 5 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |

|   | (b) i. A.             | (b) i. B.       |
|---|-----------------------|------------------|
| 6 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 7 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 8 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 9 | \( \text{x} \times \times \) | \( \text{x} \times \times \) |

|   | (b) i. C.             |
|---|-----------------------|
| 10| \( \text{x} \times \times \) | \( \text{x} \times \times \) |
| 11| \( \text{x} \times \times \) | \( \text{x} \times \times \) |

|   | (c)                   |
|---|-----------------------|
| 12| \( \text{x} \times \times \) | \( \text{x} \times \times \) |

**Fig. 1.** Illustration of the configurations of alignment considered in Lemma 1 for computing \(D(i, j)\) in the cases 1 and 2. The right-most nucleotides of the sequences \(A[1 .. i]\) and \(B[1 .. j]\) are represented using the character \(x\). The nucleotides are colored according to the type of the codon to which they belong: matching codons (M) in blue color, unmatching codons (U) in red color, inserted/deleted codons (Indel) in green color, and frameshift codons (FS) in black color. The nucleotides that appear in gray color are those belonging to codons whose type has not yet been decided. In such case, the table \(D_F\) is used in order to decide of the type of these codons later, and adjust the score accordingly.
Proof (Proof of Lemma [7]). The principle of the proof is similar to the one for the alignment of non-coding sequences [7]. For each case, the score $D(i, j)$ is the maximum score of all possible alignment configurations that are considered for this case. Here, we only describe the alignment configurations considered in the case 1 where $i (mod 3) = 0$ and $j (mod 3) = 0$. A complete proof for all the cases of the Lemma is given in Appendix. An illustration of the different configurations of alignment considered for the cases 1 and 2 is shown in Figure [4].

1. If $i (mod 3) = 0$ and $j (mod 3) = 0$, there are three cases depending on the alignment of $A[i]$ and $B[j]$.
   (a) If $A[i]$ and $B[j]$ are aligned together, there are four cases depending on whether $A[i − 2 .. i]$ and $B[j − 2 .. j]$ are grouped in the alignment or not.
      i. If both $A[i − 2 .. i]$ and $B[j − 2 .. j]$ are grouped, then $A[i − 2 .. i]$ and $B[j − 2 .. j]$ have to be aligned together, and the score of the alignment is:
         1. $s_{an}(A[i − 2 .. i], B[j − 2 .. j]) + D(i − 3, j − 3)$
      ii. If $A[i − 2 .. i]$ is grouped while $B[j − 2 .. j]$ is not grouped, then both $A[i − 2 .. i]$ and $B[j − 2 .. j]$ are FS codons ($A[i − 2 .. i]$ is a FS$^-$ codon while $B[j − 2 .. j]$ is a FS$^+$ codon). We add $2 * fs_{open \_ cost}$ to the score of the alignment, and the alignment of the nucleotides of the two FS codons can be scored independently using the scoring function $s_{an}$. There are two cases depending on the number of nucleotides from $B[j − 2 .. j]$ that are aligned with $A[i − 2 .. i]$, two or one:
         A. If $A[i − 2 .. i]$ is aligned with two nucleotides, then these nucleotides are $B[j − 1]$ and $B[j]$. There are two cases depending on the alignment of the nucleotide $B[j − 1]$ with $A[i − 1]$ or $A[i − 2]$:
            2. $s_{an}(A[i], B[j]) + s_{an}(A[i − 1], B[j − 1]) + D(i − 3, j − 2) + 2 * fs_{open \_ cost}$
            3. $s_{an}(A[i], B[j]) + s_{an}(A[i − 2], B[j − 1]) + D(i − 3, j − 2) + 2 * fs_{open \_ cost}$
         B. If $A[i − 2 .. i]$ is aligned with one nucleotide, then this single nucleotide is $B[j]$, and the score of the alignment is:
            4. $s_{an}(A[i], B[j]) + D(i − 3, j − 1) + 2 * fs_{open \_ cost}$
      iii. If $A[i − 2 .. i]$ is not grouped while $B[j − 2 .. j]$ is grouped, there are three cases that are symmetric to the three cases from (a)i.:
         5. $s_{an}(A[i], B[j]) + s_{an}(A[i − 1], B[j − 1]) + D(i − 2, j − 3) + 2 * fs_{open \_ cost}$
         6. $s_{an}(A[i], B[j]) + s_{an}(A[i − 1], B[j − 2]) + D(i − 2, j − 3) + 2 * fs_{open \_ cost}$
         7. $s_{an}(A[i], B[j]) + D(i − 1, j − 3) + 2 * fs_{open \_ cost}$
   iv. If both $A[i − 2 .. i]$ and $B[j − 2 .. j]$ are not grouped, then again both $A[i − 2 .. i]$ and $B[j − 2 .. j]$ are FS codons (both are FS$^+$ codons):
      8. $s_{an}(A[i], B[j]) + D(i − 1, j − 1) + 2 * fs_{open \_ cost}$
Lemma 2 (Filling up table $D_F$).

1. If $i \equiv 0 \pmod{3}$ and $j \equiv 0 \pmod{3}$
   $$D_F(i, j) = D(i, j)$$

2. If $i \equiv 2 \pmod{3}$ and $j \equiv 0 \pmod{3}$
   $$D_F(i, j) = \max \left\{ \begin{array}{l}
   1. \ s_{an}(A[i-1], B[j-1]) + D_F(i-2, j-3) + \text{fs open cost} \\
   2. \ s_{an}(A[i-1], B[j-2]) + D_F(i-2, j-3) + \text{fs open cost} \\
   3. \ s_{an}(A[i-2], B[j-2]) + D_F(i-2, j-1) + \text{fs open cost} \\
   4. \ s_{an}(A[i-2], B[j-1]) + D_F(i-2, j) + \text{fs open cost} \\
   5. \ s_{an}(A[i-2], B[j]) + D_F(i, j) + \text{fs open cost}
   \end{array} \right. $$

3. If $i \equiv 0 \pmod{3}$ and $j \equiv 2 \pmod{3}$, the equation is symmetric to the previous case.

4. If $i \equiv 1 \pmod{3}$ and $j \equiv 0 \pmod{3}$
   $$D_F(i, j) = \max \left\{ \begin{array}{l}
   1. \ s_{an}(A[i-1], B[j-1]) + D_F(i-1, j-3) + \text{fs extension cost} \\
   2. \ s_{an}(A[i-2], B[j-2]) + D_F(i-1, j-3) + \text{fs extension cost} \\
   3. \ s_{an}(A[i-2], B[j-1]) + D_F(i+1, j-1) + \text{fs open cost}
   \end{array} \right. $$
5. If \( i \equiv 0 \pmod{3} \) and \( j \equiv 1 \pmod{3} \), the equation is symmetric to the previous case.

The proof of Lemma 2 follows from Lemma 1. It is given in Appendix. We now present the alignment algorithm using Lemma 1 and 2 in the next theorem.

**Theorem 1.** Given two coding sequences \( A \) and \( B \) of lengths \( n \) and \( m \), a maximum score alignment between \( A \) and \( B \) can be found in time and space \( O(n \times m) \), using the following algorithm.

**Algorithm Align(\( A, B \))**

for \( i = 0 \) to \( n \) do
\[
D(i, 0) = \text{floor}\left(\frac{i}{3}\right) \times \text{gap\_cost}
\]
\[
D_F(i, 0) = D(i, 0) + \begin{cases} 
\text{san}(A[i+1], B[1]) + \text{fs\_open\_cost}, & \text{if } i \equiv 1 \pmod{3} \\
\text{san}(A[i+2], B[2]) + \text{fs\_open\_cost}, & \text{if } i \equiv 2 \pmod{3}
\end{cases}
\]

for \( j = 0 \) to \( m \) do
\[
D(0, j) = \text{floor}\left(\frac{j}{3}\right) \times \text{gap\_cost}
\]
\[
D_F(0, j) = D(0, j) + \begin{cases} 
\text{san}(A[1], B[j+1]) + \text{fs\_open\_cost}, & \text{if } j \equiv 1 \pmod{3} \\
\text{san}(A[2], B[j+2]) + \text{fs\_open\_cost}, & \text{if } j \equiv 2 \pmod{3}
\end{cases}
\]

for \( i = 0 \) to \( n \) do
for \( j = 0 \) to \( m \) do
compute \( D(i, j) \) using Lemma 1
compute \( D_F(i, j) \) using Lemma 2, if \( i \equiv 0 \pmod{3} \) or \( j \equiv 0 \pmod{3} \)

The proof of Theorem 1 is given in Appendix.

### 4 Implementation

We implemented the algorithm presented in this paper and the pairwise alignment algorithm accounting for frameshift opening penalties described in [11].

We applied both algorithms to the alignment of the examples of coding sequences Seq1, Seq2, and Seq3 described in Section 1, with the following parameters: \( \text{gap\_cost} = -1 \), \( \text{fs\_open\_cost} = -2 \), \( \text{fs\_extension\_cost} = -1 \), \( s_{aa} \) corresponding to the amino acid substitution matrix BLOSUM62, and \( s_{an} \) returning a score of +1 (resp. −1) for a match (resp. mismatch) between two nucleotides.

As predicted, the application of the algorithm from [11] to Seq1, Seq2 and Seq3 yields the same score of 72.0 for both the alignment between Seq1 and Seq2, and the alignment between Seq1 and Seq3. The present algorithm yields a score of 68.5 for Seq1 and Seq2, and a lower score of 58.0 for Seq1 and Seq3.

Using the same parameters, both algorithms were also applied to pairs of human coding sequences from paralogous genes that share a common coding subsequence translated in different frames (see [8] for a list of 470 pairs of human coding sequences presenting a frameshift event). In Appendix, the alignments obtained for the coding sequences of the protein NM_001083537 from Gene FAM86B1 and the protein NM_018172 from Gene FAM86C1 are shown. These alignments show that both coding sequences share a common prefix subsequence translated in the same frame, and a common subsequence at the end of NM_018172 translated in different frames, yielding a frameshift event. The algorithm of [11] yields a high score of 718.0 for the alignment, while the present algorithm return a score of 530 accounting for a frameshift extension length of 81 nucleotides.
5 Conclusion

We introduce a new algorithm for the pairwise alignment protein-coding sequences, accounting for translation frameshift extensions and their consequences on the modification of the protein sequences. The dynamic programming algorithm has the same asymptotic space and time complexity as the classical Needleman-Wunsch algorithm. The perspectives of this work include the evaluation of the impact of the new method on the comparison of pairs of coding sequences listed in biological databases. We also plan to study the extension of the method in the context of multiple protein-coding sequence alignment.

References

1. Federico Abascal, Rafael Zardoya, and Maximilian J Telford. Translatorx: multiple alignment of nucleotide sequences guided by amino acid translations. Nucleic acids research, page gkq291, 2010.
2. Lars Arvestad. Aligning coding dna in the presence of frame-shift errors. In Combinatorial Pattern Matching, pages 180–190. Springer, 1997.
3. Olaf RP Bininda-Emonds. transalign: using amino acids to facilitate the multiple alignment of protein-coding dna sequences. Bmc Bioinformatics, 6(1):156, 2005.
4. Fiona Cunningham, M Ridwan Amode, Daniel Barrell, et al. Ensembl 2015. Nucleic acids research, 43(D1):D662–D669, 2015.
5. Jotun Hein. An algorithm combining dna and protein alignment. Journal of Theoretical Biology, 167(2):169–174, 1994.
6. Burkhard Morgenstern. Dialign: multiple dna and protein sequence alignment at bibuserv. Nucleic acids research, 32(suppl 2):W33–W36, 2004.
7. Saul B Needleman and Christian D Wunsch. A general method applicable to the search for similarities in the amino acid sequence of two proteins. Journal of molecular biology, 48(3):443–453, 1970.
8. Kohji Okamura, Lars Feuk, Tomás Marqués-Bonet, Arcadi Navarro, and Stephen W Scherer. Frequent appearance of novel protein-coding sequences by frameshift translation. Genomics, 88(6):690–697, 2006.
9. Christian NS Pedersen, Rune Lyngsø, and Jotun Hein. Comparison of coding dna. In Combinatorial Pattern Matching, pages 153–173. Springer, 1998.
10. Kim D Pruitt, Jennifer Harrow, Rachel A Harte, et al. The consensus coding sequence (ccds) project: Identifying a common protein-coding gene set for the human and mouse genomes. Genome research, 19(7):1316–1323, 2009.
11. Vincent Ranwez, Sébastien Harispe, Frédéric Delsuc, and Emmanuel JP Douzery. Macse: Multiple alignment of coding sequences accounting for frameshifts and stop codons. PLoS One, 6(9):e22594, 2011.
12. David Sankoff and Joseph B Kruskal. Time warps, string edits, and macromolecules: the theory and practice of sequence comparison. Reading: Addison-Wesley Publication, 1983, edited by Sankoff, David; Kruskal, Joseph B., 1, 1983.
13. Temple F Smith and Michael S Waterman. Identification of common molecular subsequences. Journal of molecular biology, 147(1):195–197, 1981.
14. Rasmus Wernersson and Anders Gorm Pedersen. Revtrans: multiple alignment of coding dna from aligned amino acid sequences. Nucleic acids research, 31(13):3537–3539, 2003.
Appendix

Complete proof of Lemma 1

Proof. (Complete proof of Lemma 1). An illustration of the different configurations of alignment considered for the cases 1 and 2 of Lemma 1 in this proof is given in Figure 1. For each of the cases 1, 2, 3 and 4 of the Lemma, we first consider three cases depending on the configurations of the alignment of $A[i]$ and $B[j]$: (a) $A[i]$ and $B[j]$ are aligned together, (b) $A[i]$ is aligned with a gap, (c) $B[j]$ is aligned with a gap.

1. If $i \pmod{3} = 0$ and $j \pmod{3} = 0$, then $A[i]$ and $B[j]$ are the last nucleotides of two codons $A[i-2..i]$ and $B[j-2..j]$. There are three cases depending on the alignment of $A[i]$ and $B[j]$.
   (a) If $A[i]$ and $B[j]$ are aligned together, there are four cases depending on whether $A[i-2..i]$ and $B[j-2..j]$ are grouped in the alignment or not.
   i. If both $A[i-2..i]$ and $B[j-2..j]$ are grouped, then $A[i-2..i]$ and $B[j-2..j]$ have to be aligned together, and the score of the alignment is:
      1. $s_{aa}(A[i-2..i], B[j-2..j]) + D(i-3, j-3)$
   ii. If $A[i-2..i]$ is grouped while $B[j-2..j]$ is not grouped, then both $A[i-2..i]$ and $B[j-2..j]$ are FS codons ($A[i-2..i]$ is a FS- codon while $B[j-2..j]$ is a FS+ codon). We add $2 \times \text{fs}_\text{open}_\text{cost}$ to the score of the alignment, and the alignment of the nucleotides of the two FS codons can be scored independently using the scoring function $s_{an}$. There are two cases depending on the number of nucleotides from $B[j-2..j]$ that are aligned with $A[i-2..i]$, two or one:
      A. If $A[i-2..i]$ is aligned with two nucleotides, then these nucleotides are $B[j-1]$ and $B[j]$. There are two cases depending on the alignment of the nucleotide $B[j-1]$ with $A[i-1]$ or $A[i-2]$:
         2. $s_{an}(A[i], B[j]) + s_{an}(A[i-1], B[j-1]) + D(i-3, j-2) + 2 \times \text{fs}_\text{open}_\text{cost}$
         3. $s_{an}(A[i], B[j]) + s_{an}(A[i-2], B[j-1]) + D(i-3, j-2) + 2 \times \text{fs}_\text{open}_\text{cost}$
      B. If $A[i-2..i]$ is aligned with one nucleotide, then this single nucleotide is $B[j]$, and the score of the alignment is:
         4. $s_{an}(A[i], B[j]) + D(i-3, j-1) + 2 \times \text{fs}_\text{open}_\text{cost}$
   iii. If $A[i-2..i]$ is not grouped while $B[j-2..j]$ is grouped, there are three cases that are symmetric to the three cases from (a)ii.:
      5. $s_{an}(A[i], B[j]) + s_{an}(A[i-1], B[j-1]) + D(i-2, j-3) + 2 \times \text{fs}_\text{open}_\text{cost}$
      6. $s_{an}(A[i], B[j]) + s_{an}(A[i-1], B[j-2]) + D(i-2, j-3) + 2 \times \text{fs}_\text{open}_\text{cost}$
      7. $s_{an}(A[i], B[j]) + D(i-1, j-3) + 2 \times \text{fs}_\text{open}_\text{cost}$
iv. If both $A[i - 2 .. i]$ and $B[j - 2 .. j]$ are not grouped, then again both $A[i - 2 .. i]$ and $B[j - 2 .. j]$ are FS codons (both are $FS^+$
codons):
8. $s_{an}(A[i], B[j]) + D(i - 1, j - 1) + 2*fs_{open}_{cost}$

(b) If $A[i]$ is aligned with a gap, then the codon $A[i - 2 .. i]$ is a FS codon
($FS^-$ or $FS^+$). We must add $fs_{open}_{cost}$ to the score of the alignment. There are two cases depending on whether $A[i - 2 .. i]$ is grouped in the
alignment or not.

i. If $A[i - 2 .. i]$ is grouped, then there are three cases depending on
the number of nucleotides from $B[j - 2 .. j]$ that are aligned with
$A[i - 2 .. i]$, two, one, or zero.

A. If $A[i - 2 .. i]$ is aligned with two nucleotides, then these
nucleotides are $B[j - 1]$ and $B[j]$. The score of the alignment is:
9. $s_{an}(A[i - 1], B[j]) + s_{an}(A[i - 2], B[j - 1]) + D_F(i - 3, j - 2) + fs_{open}_{cost}$

B. If $A[i - 2 .. i]$ is aligned with one nucleotide, then this single
nucleotide is $B[j]$. There are two cases depending on the alignment
of the nucleotide $B[j]$ with $A[i - 1]$ or $A[i - 2]$:
10. $s_{an}(A[i - 1], B[j]) + D(i - 3, j - 1) + 2*fs_{open}_{cost}$
11. $s_{an}(A[i - 2], B[j - 1]) + D_F(i - 3, j - 1) + fs_{open}_{cost}$

C. If $A[i - 2 .. i]$ is aligned with zero nucleotide, then the codon
$A[i - 2 .. i]$ is entirely deleted. The score of the alignment is:
12. $gap_{cost} + D(i - 3, j)$

ii. If $A[i - 2 .. i]$ is not grouped, then the codon $A[i - 2 .. i]$ is a $FS^+$
codon, and the score of the alignment is:
13. $D(i - 1, j) + fs_{open}_{cost}$

(c) If $B[i]$ is aligned with a gap, there are five cases that are symmetric
to the five cases from (b):
14. $s_{an}(A[i], B[j - 1]) + s_{an}(A[i - 1], B[j - 2]) + D_F(i - 2, j - 3) + fs_{open}_{cost}$
15. $s_{an}(A[i], B[j - 1]) + D(i - 1, j - 3) + 2*fs_{open}_{cost}$
16. $s_{an}(A[i], B[j - 2]) + D_F(i - 1, j - 3) + fs_{open}_{cost}$
17. $gap_{cost} + D(i, j - 3)$
18. $D(i, j - 1) + fs_{open}_{cost}$

2. If $i (mod 3) = 0$ and $j (mod 3) \neq 0$, then $A[i]$ is the last nucleotide of a
codon $A[i - 2 .. i]$ and $B[j]$ is not the last nucleotide of a codon. There are
three cases depending on the alignment of $A[i]$ and $B[j]$.

(a) If $A[i]$ and $B[j]$ are aligned together, there are two cases depending
on whether $A[i - 2 .. i]$ is grouped in the alignment or not.

i. If $A[i - 2 .. i]$ is grouped, there are three cases depending on
the number of nucleotides from $B$ that are aligned with $A[i - 2 .. i]$, three, two, or one:
A. If $A[i - 2 .. i]$ is aligned with three nucleotides, then these
nucleotides are $B[j]$, $B[j - 1]$, and $B[j - 2]$. We are in the case
of an unmatching (U) codon. The score of the alignment is then:
1. $s_{an}[A[i-2..i],B[j-2..j]] + D_F(i-3,j-3) + f_s\text{ extension cost}$
2. $s_{an}(A[i],B[j])$ if $j - 1 (mod 3) \neq 0$

B. If $A[i-2..i]$ is aligned with two nucleotides, then these nucleotides are $B[j]$ and $B[j-1]$. $A[i-2..i]$ is a FS$^+$ codon. There are two cases depending on the alignment of $B[j-1]$ with $A[i-1]$ or $A[i-2]$. In both cases, if $j - 1 (mod 3) = 0$, then $j - 1$ is the last nucleotide of a codon. We should then make adjustments in order to account for the type of this codon (FS$^+$, or unknown type for now):
3. $s_{an}(A[i],B[j]) + s_{an}(A[i-1],B[j-1]) + D(i-3,j-2) + f_s\text{ open cost}$ if $j - 1 (mod 3) = 0$
4. $s_{an}(A[i],B[j]) + s_{an}(A[i-2],B[j-1]) + D(i-3,j-2) + f_s\text{ open cost}$ if $j - 1 (mod 3) = 0$

C. If  $A[i-2..i]$ is aligned with one nucleotide, then $A[i-2..i]$ is a FS$^-$ codon. The score of the alignment is:
5. $s_{an}(A[i],B[j]) + D(i-2,j-1) + f_s\text{ open cost}$

(b) If $A[i]$ is aligned with a gap, there are two cases depending on whether $A[i-2..i]$ is grouped in the alignment or not.

i. If $A[i-2..i]$ is grouped, there are three cases depending on the number of nucleotides from $B$ that are aligned with $A[i-2..i]$, two, one, or zero.

A. If $A[i-2..i]$ is aligned with two nucleotides, then these nucleotides are $B[j]$ and $B[j-1]$. $A[i-2..i]$ is a FS$^+$ codon. If $j - 1 (mod 3) = 0$, then $j - 1$ is the last nucleotide of a codon. We should make adjustments in order to account for the fact no type has yet been decided for this codon.
6. $s_{an}(A[i-1],B[j]) + s_{an}(A[i-2],B[j-1]) + D_F(i-3,j-2) + f_s\text{ open cost}$ if $j - 1 (mod 3) = 0$

B. If $A[i-2..i]$ is aligned with one nucleotide, then this single nucleotide is $B[j]$. $A[i-2..i]$ is a FS$^-$ codon. There are two cases depending on the alignment of $B[j]$ with $A[i-1]$ or $A[i-2]$;
7. $s_{an}(A[i-1],B[j]) + D(i-3,j-1) + f_s\text{ open cost}$
8. $s_{an}(A[i-2],B[j]) + D(i-3,j-1) + f_s\text{ open cost}$

C. If $A[i-2..i]$ is aligned with zero nucleotides, the codon $A[i-2..i]$ is entirely deleted:
9. $\text{gap cost} + D(i-3,j)$

ii. If $A[i-2..i]$ is not grouped
10. $D(i-1,j) + f_s\text{ open cost}$

(c) If $B[j]$ is aligned with a gap, then the score of the alignment is:
11. $D(i,j-1)$
3. If $i \mod 3 \neq 0$ and $j \mod 3 = 0$, the proof is symmetric to the previous proof for 2.

4. If $i \mod 3 \neq 0$ and $j \mod 3 \neq 0$, there are three cases depending on the alignment of $A[i]$ and $B[j]$.
   
   \( (a) \) If $A[i]$ and $B[j]$ are aligned together, the score of the alignment is:
   
   1. $s_{an}(A[i], B[j]) + D(i - 1, j - 1)$
   
   \( (b) \) If $A[i]$ is aligned with a gap, the score of the alignment is:
   
   2. $D(i - 1, j)$
   
   \( (c) \) If $B[j]$ is aligned with a gap, the score of the alignment is:
   
   3. $D(i, j - 1)$

Proof of Lemma 2

Proof (Proof of Lemma 2). The proof follows from Lemma 1.

1. If $i \mod 3 = 0$ and $j \mod 3 = 0$, this case is trivial.

2. If $i \mod 3 = 2$ and $j \mod 3 = 0$, then $i + 1 \mod 3 = 0$ and $j + 1 \mod 3 = 1 \neq 0$. The five cases follow from the application of Lemma 1. Case 2 for computing $D(i + 1, j + 1)$, and by keeping only the cases where $A[i + 1]$ and $B[i + 1]$ are aligned together (cases 1, 2, 3, 4, 5 among the 11 cases). However, in each of the cases, we must subtract half of the score of aligning $B[i + 1]$ with $A[i + 1]$ ($\frac{s_{an}(A[i+1], B[i+1])}{2}$), because this score will be added subsequently.

3. If $i \mod 3 = 0$ and $j \mod 3 = 2$, the proof is symmetric to the previous case.

4. If $i \mod 3 = 1$ and $j \mod 3 = 0$, then $i + 2 \mod 3 = 0$ and $j + 2 \mod 3 = 2 \neq 0$. Here again, the three cases follow from the application of Lemma 1. Case 2 for computing $D(i + 2, j + 2)$, and by keeping only the cases where $A[i + 1], B[i + 1], A[i + 1 = 2], B[i + 2]$ can be aligned together (cases 1, 2, 5 among the 11 cases). However, in each of the cases, we must subtract half of the scores of aligning $B[i + 2]$ with $A[i + 2]$, and aligning $B[i + 1]$ with $A[i + 1]$ ($\frac{s_{an}(A[i+2], B[i+2])}{2}, \frac{s_{an}(A[i+1], B[i+1])}{2}$), because these scores will be added subsequently.

5. If $i \mod 3 = 0$ and $j \mod 3 = 1$, the proof is symmetric to the previous case.

Proof of Theorem 1

Proof (Proof of Theorem 1). The proof relies on two points: (1) The algorithm computes the maximum score of an alignment between $A$ and $B$, and (2) the algorithm runs with an $O(nm)$ time and space complexity.

(1) The validity of the algorithm, i.e. the facts that it fills the cells of the tables $D, D_F$ according to Definition 1, follows from five points.
The initialization of the tables is a direct consequence of Definition 1, Lemmas 1 and 2.

The couples \((i, j)\) of prefixes of \(A\) and \(B\) that need to be considered in the algorithm are all the possible couples for \(D(i, j)\), and only the couples such that \(i(\text{mod} \ 3) = 0\) or \(j(\text{mod} \ 3) = 0\) for \(D_F(i, j)\) (see all the cases in which the table \(D_F\) is used in Lemmas 1 (7 cases) and 2 (3 cases)).

The couples \((i, j)\) of prefixes of \(A\) and \(B\) are considered in increasing order of length, and \(D[i, j]\) is computed before \(D_F[i, j]\) in the cases where \(i(\text{mod} \ 3) = 0\) or \(j(\text{mod} \ 3) = 0\).

A backtracking of the algorithm allows to find a maximum score alignment between \(A\) and \(B\).

(2) The time and space complexity of the algorithm is a direct consequence of the number of cells of the tables \(D\) and \(D_F\), \(2 \times (n + 1 \times m + 1)\). Each cell is filled in constant time.

Alignment of coding sequences NM_001083537 and NM_018172 using a previously published method [11] and the present method

Translations of NM_001083537 and NM_018172 into protein sequences

\[\text{NM}_001083537\]
MAPEENAGTEELLQGFERRFLAVRTLRSFPWQSLEAKLRDSSDSELLRDLQKTVRHPVC
VKHPSVYACWFLSELIKSSSGGSVTLSKSTAIISSHGTTLGVVTDAALYLAEAEWENPA
AFINRTVLEGSGAGLCTLAICKMCRPRAYIFSPHSRVLQLEQLRGNVLLNLSELEAISTG
NLDSRPRVTAVQLSWDVAMVHQLSAFQPDDVIAADVLYCPEAISVLSVGLQRLAACRENKR
APEVYVAFTVRNRPETCQLFTTELGRDGRWEAEAAHIDQKLFYPYEHELEAMMLNLT*

\[\text{NM}_018172\]
MAPEENAGSELLLQSFKRRLAARALRSLFRRWSLEAKLRDSSDSELLRDLQKHEAVHTE
PLDELYEVVLVETLMAKESTQTGHRSYLTCTCIAQKPCRWSGCWGLPAGSTSGLLNSTW
PLPSATQRACSCPSYAGLSDGKRLIMTRNCFPATESTWRWQS*

Score obtained with previously published method : 718.0

A!!TGCGCCCAGGAGAAACCGGGGACCGAATCTCTGCTGACAGGTGTGTGAGGCGGCT
A!!TGCGCCCAGGAGAAACCGGGGACCGAATCTCTGCTGACAGGTGTGTGAGGCGGCT

TCTCTG---CGTGCGACAACCTCGCGCTCTTTC1CCC---TGGCGAGAGCCTAGAGGCAAG
TCTCTGCGACCCG---GCGCCCAGCCGCTCTTTC1!!CCGC!!TGCGAAGACCTAGAGGCAAG
TTAAGAGACT! CATCAGATTCTGAGCTGTGCGGGATATTGGCGAGAACAGACGCTGAGGC
TTAAGAGACT! CATCAGATTCTGAGCTGTGCGGGATATTGGCGAGAACAGACGCTGAGGC
Score obtained with present method: 530.0
TCATCGAGATTCTGAGCTGCTGCGGGATATTTTGCAAGAACAGACTGTGAGGCATCCTGTGT
TCATCAGATTCTGAGCTGCTGCGGGATATTTTGCAAGAACAGACTGTGAGGCATCCTGTGT

---AACAC-------------------------GAG---

TCCTCAGGAGGCTCACTCACAATCTCCAAGAGCAAGACATCTCTCAGAACTCATCAAAAG
---AAGCAC------------------------------------GAG---

GGCTGTGTCACATGGGATGCCCTCGTCTAAGCAGAGACATCGAGAACCAGGGA
GCTTCTTTGAGTTGCTGTCACLGCTGACGAGAGACAGCTAGCATGACCAGA
---CTG-------------------------AAG------------

ATGTGCAAGATGTGAGCCCGGGCGGCAACTACATCTTCAGACGACCCCTCAAGCTTCCTC
---------ATG------------------------AAG---

AACTTAGACAGCCCGGGGAAGGACTGGCCCTATTAGAGGCAGACATCACTGGC
---------AAG-------------------------------GAG---

AACTTAGAAGCAGCGGTACAGTGGCCAGCTCTGCAGATTGCAGAATGGGCCATCGAGAACCCGGCA
CCTCTGGAGGCTACGTGGCCTTTACCGTCCGCAACCCAGAGACGTG---CAGCTGTTCA
GCTCCTCAATTCTACAGTGGCTCTTACCGTCCGCAACCCAGAGATGTCAAACCTCTACG
---------TCC---------------ACC-----CAG--------------------------------GAG---

CACCTCTCTGTGAGCCAGATGCTCTGCTGAGGAGGCTGTGATTTGCCCAGA
CGG----AGC-----TAT----------------TTGCT---GACGTGCTGTATTGCCCAGAA
GCCATCGTGTCGCTGCGGGGTCCTGCAGAGGCTGGCTGCCTGCCGGGAGCACAAGCGG
GCCATCGTGTCGCTGCGGGGTCCTGCAGAGGCTGGCTGCCTGCCGGGAGCACCAGCGG
GCTCCTGAGGTCTACGTGGCCTTTACCGTCCGCAACCCAGAGACGTGC--CAGCTGTTCA
GCTCCTCAATTCTACAGTGGCTCTTACCGTCCGCAACCCAGAGATGTCAAACCTCTACG
---------TCC---------------ACC-----CAG--------------------------------GAG---

AAACTTTCTTCCTACAGGAGCCTTGGAGATGGCAATGCTGAACCTCACACTGTAG
AAACTTCTTCCTACAGGAGCCTTGGAGATGGCAATGCTGAACCTCACACTGTAG

---AACAC-------------------------GAG---

AAACTTTCTTCCTACAGGAGCCTTGGAGATGGCAATGCTGAACCTCACACTGTAG
AAACTTTCTTCCTACAGGAGCCTTGGAGATGGCAATGCTGAACCTCACACTGTAG

---AACAC-------------------------GAG---

AAACTTTCTTCCTACAGGAGCCTTGGAGATGGCAATGCTGAACCTCACACTGTAG
AAACTTTCTTCCTACAGGAGCCTTGGAGATGGCAATGCTGAACCTCACACTGTAG