IDENTIFICATION OF PROTEIN CODING GENES IN GENOMES WITH STATISTICAL FUNCTIONS BASED ON THE CIRCULAR CODE

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ABSTRACT

A new statistical approach using functions based on the circular code classifies correctly more than 93 % of bases in protein (coding) genes and non-coding genes of human sequences. Based on this statistical study, a research software called "Analysis of Coding Genes" (ACG) has been developed for identifying protein genes in the genomes and for determining their frame. Furthermore, the software ACG also allows an evaluation of the length of protein genes, their position in the genome, their relative position between themselves, and the prediction of internal frames in protein genes.

1. INTRODUCTION

The concept of code "without commas", introduced by Crick *et al.* (1957) for the protein (coding) genes, is a code readable in only one out of three frames. Such a theoretical code without commas, called circular code in the theory of codes (e.g., Béal, 1993; Berstel & Perrin, 1985), is a particular set *X* of trinucleotides such that a concatenation (a series) of trinucleotides of *X* leads to sequences that cannot be decomposed in another frame with a concatenation of trinucleotides of *X*.

For example, suppose that *X* is the following set of trinucleotides: *X*={AAC, AAT, ACC, ATC, ATT, CAG, CTC, CTG, GAA, GAC, GAG, GAT, GCC, GGC, GGT, GTA, GTC, GTT, TAC, TTC}. Some trinucleotides of *X* are concatenated randomly, for example as follows:

…CAG,GCC,TTC,AAT,ACC,ACC,CAG,GAA,GAG,GTA,ATT,ACC,AAT,GTA,AAC,TAC,TTC,ACC,ATC…

The commas between the trinucleotides show the frame of construction (reading frame in biology). Suppose now that the commas are “lost”, leading to the sequence:

…CAGGCCTTCAATACCACCCAGGAAGAGGTAATTACCAATGTAAACTACTTCACCATC…

The problem is to retrieve the original frame of construction. There are 3 obvious possibilities:

…C,AGG,CCT,TCA,ATA,CCA,CCC,AGG,AAG,AGG,TAA,TTA,CCA,ATG,TAA,ACT,ACT,TCA,CCA,TC…

…CA,GGC,CTT,CAA,TAC,CAC,CCA,GGA,AGA,GGT,AAT,TAC,CAA,TGT,AAA,CTA,CTT,CAC,CAT,C…

…CAG,GCC,TTC,AAT,ACC,ACC,CAG,GAA,GAG,GTA,ATT,ACC,AAT,GTA,AAC,TAC,TTC,ACC,ATC…

If the set *X* of trinucleotides is a circular code, then there is an unique solution:

…CAG,GCC,TTC,AAT,ACC,ACC,CAG,GAA,GAG,GTA,ATT,ACC,AAT,GTA,AAC,TAC,TTC,ACC,ATC…

This unique solution is obtained by choosing a window (sufficiently large) in any position in the sequence and then verifying the belonging of the trinucleotides of the window to *X*:

…CAGGCCTTCAATACCACCCAGGAAG⌊~~AGG~~,TAATTACCAATGTAAACTACTTCACCATC…

…CAGGCCTTCAATACCACCCAGGAAG⌊A,GGT,AAT,TAC,~~CAA~~,TGTAAACTACTTCACCATC…

…CAGGCCTTCAATACCACCCAGGAAG⌊AG,GTA,ATT,ACC,AAT,GTA,AAC,TAC,TTC,ACC,ATC,…

The first decomposition proposed is rejected immediately as the first trinucleotide AGG in the window does not belong to *X*. The second decomposition proposed is rejected with a window of 13 nucleotides. Indeed, the first nucleotide A in the window may belong to several trinucleotides of *X*, e.g., GTA. The trinucleotides GGT, AAT, and TAC following A belong to *X*. The next trinucleotide CAA does not belong to *X* as the 13th nucleotide A (from the beginning of the window) differs from the unique possibility G of CAG belonging to *X*. The third decomposition is the original one as all the trinucleotides in the window belong to *X* and the original decomposition of the sequence is deduced automatically.

Such a code was proposed by Crick *et al.* (1957) in order to explain how the reading of a series of nucleotides in the protein genes could code for the amino acids constituting the proteins. The 2 problems stressed were: why are there more trinucleotides than amino acids and how to choose the correct reading frame? Crick *et al.* (1957) proposed that only 20 among 64 trinucleotides code for the 20 amino acids. However, the determination of a set of 20 trinucleotides forming a circular code *X* depends on a great number of constraints:

(i) A trinucleotide with identical nucleotides (AAA, CCC, GGG or TTT) must be excluded from such a code. Indeed, the concatenation of AAA with itself does not allow the retrieval of the reading (original) frame as there are 3 possible decompositions: ...AAA,AAA,AAA,..., ...A,AAA,AAA,AA... and ...AA,AAA,AAA,A...

(ii) Two trinucleotides related to circular permutation, e.g., ATC and TCA, must be excluded from such a code. Indeed, the concatenation of ATC with itself does not allow the retrieval of the reading (original) frame as there are 2 possible decompositions: …ATC,ATC,ATC,… and …A,TCA,TCA,TC…
Therefore, by excluding AAA, CCC, GGG and TTT and by gathering the 60 remaining trinucleotides in 20 classes of 3 trinucleotides so that, in each class, the 3 trinucleotides are deduced from each other by circular permutations, e.g., ATC, TCA, and CAT, a circular code, has only one trinucleotide per class and therefore contains at most 20 trinucleotides (maximal circular code). This trinucleotide number is identical to the amino acid number suggesting a circular code assigning one trinucleotide per amino acid.

No set of 20 trinucleotides leading to a circular code has been found at this time. Furthermore, the 2 discoveries that the trinucleotide TTT, an "excluded" trinucleotide in the concept of circular code, codes for phenylalanine (Nirenberg & Matthaei, 1961) and that the protein genes are placed in the reading frame with a particular trinucleotide, namely the start trinucleotide ATG, have led to giving up the concept of circular code on the alphabet {A,C,G,T}. For several biological reasons, in particular the interaction between mRNA and tRNA, the concept of circular code has been resumed subsequently regarding the alphabet {R,Y} (R=purine=A or G, Y=pyrimidine=C or T) with 2 trinucleotide models for the primitive protein genes: RRY (Crick *et al.*, 1976) and RNY (N=R or Y) (Eigen & Schuster, 1978).

Unexpectedly, a maximal circular code has been identified recently in the protein genes of both eukaryotes and prokaryotes on the alphabet {A,C,G,T} (Arquès & Michel, 1996). This circular code has been obtained by 2 methods:
(i) by computing the occurrence frequencies of the 64 trinucleotides AAA,...,TTT in the 3 frames of protein genes and then, by assigning each trinucleotide to the frame associated with its highest frequency (Arquès & Michel, 1996);
(ii) by computing the 12288 (3×64^2) autocorrelation functions analysing the probability that a trinucleotide in any frame occurs any i bases N after a trinucleotide in a given frame of protein genes and then, by classifying these autocorrelation functions according to their modulo 3 periodicity for deducing a frame for each trinucleotide (Arquès & Michel, 1997a).
The maximal circular code identified is the set $X_0 = \{$AAC,AAT,ACC,ATC,ATT,CAG,CTC,CTG,GAA,GAC, GAG,GAT,GCC,GGC,GGT,GTA,GTC,GTT,TAC,TTC$\}$ of 20 trinucleotides in frame 0 of protein genes (reading frame). Furthermore, the 2 sets X_1 and X_2 of 20 trinucleotides identified in the frames 1 and 2, respectively, (frames 1 and 2 being the frame 0 shifted by 1 and 2 nucleotides respectively in the 5'-3' direction) by these 2 methods, are also maximal circular codes (Table 1a). These 3 circular codes have several important properties:
(i) circularity: X_0 generates X_1 by one circular permutation and X_2 by another circular permutation (1 and 2 circular permutations of each trinucleotide of X_0 lead to the trinucleotides of X_1 and X_2 respectively) (Table 1b).
(ii) complementarity: X_0 is self-complementary (10 trinucleotides of X_0 are complementary to the 10 other trinucleotides of X_0) and, X_1 and X_2 are complementary to each other (the 20 trinucleotides of X_1 are complementary to the 20 trinucleotides of X_2) (Table 1c). Note that this property is also verified with $T_0 = X_0 \bigcup \{\text{AAA,TTT}\}$, $T_1 = X_1 \bigcup \{\text{CCC}\}$ and $T_2 = X_2 \bigcup \{\text{GGG}\}$ (Table 1c).

(iii) rarity: the occurrence probability of X_0 is equal to 6×10^{-8}. As there are 20 classes of 3 trinucleotides (see above), the number of potential circular codes is 3^{20}=3486784401. The computed number of complementary circular codes with 2 shifted circular codes (called C^3 codes), such as X_0, is 216. Therefore, its probability is $216/3^{20}=6\times10^{-8}$.

(iv) flexibility:

- the lengths of the minimal windows to automatically retrieve the frames 0, 1, and 2 with the 3 circular codes X_0, X_1, and X_2 respectively, are all equal to 13 nucleotides and represent the largest window length among the 216 C^3 codes.
- the frequency of misplaced trinucleotides in the shifted frames is equal to 24.6%. If the trinucleotides of X are concatenated randomly, for example as follows:

…GAA,GAG,GTA,GTA,ACC,AAT,GTA,CTC,TAC,TTC,ACC,ATC…

then, the trinucleotides in frame 1:

…G,AAG,AGG,TAG,TAA,CCA,ATG,TAC,TCT,ACT,TCA,CCA,TC…

and the trinucleotides in frame 2:

…GA,AGA,GGT,AGT,AAC,CAA,TGT,ACT,CTA,CTT,CAC,CAT,C…

belong mainly to X_1 and X_2, respectively. A few trinucleotides are misplaced in the shifted frames. With this example, in frame 1, 9 trinucleotides belong to X_1, 1 trinucleotide (TAC) to X_0 and 1 trinucleotide (TAA) to X_2. In frame 2, 8 trinucleotides belong to X_2, 2 trinucleotides (GGT, AAC) to X_0 and 1 trinucleotide (ACT) to X_1. By computing exactly, the average frequencies of misplaced trinucleotides in frame 1 are 11.9 % for X_0 and 12.7 % for X_2. In frame 2, the average frequencies of misplaced trinucleotides are 11.9 % for X_0 and 12.7 % for X_1. The complementarity property explains on the one hand that the frequency equality of X_0 in frames 1 and 2 and on the other hand, the frequency equality of X_2 in frame 1 and X_1 in frame 2. The sum of percentages of misplaced trinucleotides in frame 1 (X_0 and X_2) is equal to the sum of percentages of misplaced trinucleotides in frame 2 (X_0 and X_1) and is equal to 24.6 %. This value is close to the highest frequency (27.9 %) of misplaced trinucleotides among the 216 C^3 codes.

- the 4 types of nucleotides occur in the 3 trinucleotide sites with the 3 circular codes X_0, X_1, and X_2 (Table 1a).

(v) evolutionary: an evolutionary analytical model at 3 parameters (p,q,t) based on an independent mixing of the 20 trinucleotides of X_0 with equiprobability (1/20) followed by $t\approx4$ substitutions per trinucleotide according to the proportions $p\approx0.1$, $q\approx0.1$ and $r=1-p-q\approx0.8$ in the 3 trinucleotide sites respectively, retrieves the frequencies of X_0, X_1, and X_2 observed in the 3 frames of protein genes.

The proof that X_0, X_1, and X_2 are circular codes, the detailed explanation of the properties (i-iv) and the different biological consequences, in particular on the 2-letter genetic alphabets, the genetic code and the amino acid frequencies in proteins, are given in Arquès & Michel (1996, 1997a). The property (v) is described in Arquès *et al.* (1998, 1999).

Note: a non-complementary circular code has been identified recently in the mitochondrial protein genes (Arquès & Michel, 1997b).

As the circular code is a strong structural property of protein genes, different statistical functions based on the circular code are investigated in this paper in order to discriminate between coding and non-coding genes. Indeed, the sets of 20 trinucleotides based on a circular code, i.e., the 216 C^3 codes and in particular X_0, X_1, and X_2, have a lesser number of misplaced trinucleotides in the shifted frames compared to the vast majority of sets without particular property. This low number implies that the 3 circular codes X_0, X_1, and X_2 can clearly be associated with the 3 frames 0, 1, and 2 respectively (detailed in method).

After having validated this statistical approach with the human sequences from the EMBL database, research software has been developed for identifying protein genes in genomes and for determining their frame. Furthermore, this software also allows an evaluation of the length of protein genes, their position in the genome, their relative position between themselves, and the prediction of internal frames. These possibilities are presented with 5 examples taken from human chromosomes: a large protein gene, a complementary protein gene, a series of 5 exons, a protein gene with 4 internal frames, and a possible coding region in the human DNA sequence. An example with a prokaryotic genome is also given.

2. METHOD

2.1. Introduction

The method developed is based on a strong structural property of protein genes, i.e., the circular code, and in particular its properties of circularity and complementarity. This method differs from the classical methods, such as the codon usage methods and the HMM methods, at least for the following reasons:

(i) The circular code is observed in protein genes of eukaryotes as well as of prokaryotes and is not found in the non-coding genes (Arquès *et al.*, 1998). Therefore, a method based on this circular code can be applied independently of the type of eukaryotic/prokaryotic organism under investigation. In contrast, the codon usage methods use codon frequencies that depend on the species and the functional classes of protein genes (see e.g., Karlin *et al.*, 1998).

(ii) The circular code X_0 (resp. X_1 and X_2) contains the 20 codons having a preferential occurrence in the frame 0 (resp. 1 and 2). It is important to stress that the set X_0, for example, does not necessarily represent the common codons in frame 0, i.e., the 20 codons having the highest frequencies in frame 0 (see a few examples in Table 2).

(iii) The 216 C^3 codes have a low number of misplaced trinucleotides in the shifted frames, 27.9 % in the worst case and 24.6 % for X_0. This number is close to 2/3≈66.6 % with the vast majority of trinucleotide sets without particular property. Indeed, by excluding AAA, CCC, GGG, and TTT, there is one chance out of 3 to observe, for example, a codon of X_1 in frame 1, i.e., 2 chances out of 3 to observe a codon of X_0 or X_2 in frame 1. In summary, the method developed according to the circular code allows to associate clearly the 3 sets of trinucleotides X_0, X_1, and X_2 with the 3 frames 0, 1 and 2 respectively of protein genes.

(iv) The complementarity property of these 3 sets X_0, X_1, and X_2 is used for identifying protein genes on the direct strand but also on the complementary strand (see the definition of the 4 functions below).

(v) The method developed is based on the global probabilities of X_0, X_1, and X_2 and not on the individual codon probabilities that are used in the codon usage methods.

2.2. Definition of statistical functions

Let *t* be a trinucleotide in the set {AAA,...,TTT} (64 trinucleotides). Let *F* be a population with $m(F)$ sequences *S*. Each sequence *S* has a base length $l(S)$. Let w_i be a window of *n* trinucleotides starting at the base position *i*, $i = 1,...,l(S)-3n+1$, in a sequence *S* of *F*, i.e., $w_i = t_1...t_n$ where t_j is the *j*th trinucleotide in the window w_i. Let T_g, $g \in \{0,1,2\}$, be the 3 subsets of trinucleotides constituting the 3 circular codes in the protein coding genes of eukaryotes and prokaryotes, T_0 in the open reading frame (frame 0) and, T_1 and T_2, in the shifted frames 1 and 2 respectively (Table 1a). In a given window w_i, the function $\delta_g(t_j) = \begin{cases} 1 & \text{if } t_j \in T_g \\ 0 & \text{if } t_j \notin T_g \end{cases}$ determines whether or not if the trinucleotide t_j at the position *j* in w_i belongs to T_g with $g \in \{0,1,2\}$. Next, the occurrence frequency $P(T_g,w_i)$ of a subset T_g in w_i, is $P(T_g,w_i) = \sum_{j=1}^{n} \delta_g(t_j) / n$ where *n* is the total number of trinucleotides in the window w_i.

Several statistical functions based on the properties of the circular code, are defined:

$$F_1(i) = P(T_0,w_i) \quad (1)$$

$$F_2(i) = P(T_0,w_i) - P(T_2,w_i) \quad (2)$$

$$F_3(i) = \frac{21}{22} 2P(T_0,w_i) \Big/ \left(P(T_1,w_i) + P(T_2,w_i)\right) \quad (3)$$

$$F_4(i) = \sum_{j=0}^{2} P(T_j,w_{i+j}) \quad (4)$$

These 4 statistical functions use different properties of the circular code, in particular the properties of circularity and complementarity.

The function F_1 is the simplest, and is based on the circular code X_0 (extended to T_0) in each window w_i. In a protein gene, $F_1(i)$ associated with the reading frame of the sequence (i.e., w_i in reading frame and therefore w_{i+1} and w_{i+2} in the shifted frames 1 and 2, respectively) is in general greater than $F_1(i+1)$ and $F_1(i+2)$ as the occurrence probability of T_0 is by definition maximum in the reading frame (see point (ii) of Section 2.1 explaining the misplaced trinucleotides).

The function F_2 considers the 2 circular codes X_0 and X_2 (extended to T_0 and T_2). The probability difference $P(T_0,w_i) - P(T_2,w_i)$ is maximum among the 18 possible probability differences in the 3 frames. Indeed, the average probabilities of T_0, T_1, and T_2 in the frame 0 (resp. 1, 2) of protein genes are 49 % (resp. 26.5 %, 32 %), 28.5 % (resp. 43 %, 23 %), and 22.5 % (resp. 30.5 %, 45 %) (Arquès *et al.*, 1998). By consequence, the maximum probability difference in frame 0 (resp. 1, 2) is 26.5 % with $\mathrm{Prob}(T_0) - \mathrm{Prob}(T_2)$ (resp. 16.5 % with $\mathrm{Prob}(T_1) - \mathrm{Prob}(T_0)$, 22 % with $\mathrm{Prob}(T_2) - \mathrm{Prob}(T_1)$).

The functions F_3 and F_4 are based on the 3 circular codes X_0, X_1, and X_2 (extended to T_0, T_1, and T_2). The function F_3 tests a ratio that is maximum in the reading frame. The functions F_1, F_2, and F_3 favor the

circular code X_0 characterizing the reading frame, while the function F_4 considers the 3 circular codes in their 3 associated frames.
Finally, as T_0 is self-complementary, and as T_1 and T_2 are complementary to each other, the 4 functions have a similar behaviour on both strands.

These statistical functions are represented by curves as follows. As the value of $F_k(i)$ is a mean value computed from the bases of the window w_i, i.e., the bases $i, i+1, ..., i+3n-1$, the point associated with $F_k(i)$ is represented graphically in the abscissa by the base position $i + \lfloor 3n/2 \rfloor$ (greatest integer less or equal) and in the ordinate by $F_k(i)$. As the protein coding genes have 3 frames, a function F_k is represented by 3 curves where the points are joined modulo 3: by convention, the curve C_j, $j = 0,1,2$, joins the points associated with $F_k(i)$ with $i = (j+1) \bmod 3$, i.e., the curve C_0 (resp. C_1, C_2) joins the points in base position 1 (resp. 2, 0) modulo 3 (Fig. 1a) and therefore is related to the base position in frame 0 (resp. 1, 2) determined from the beginning of the sequence.

The 4 functions defined above are extended to the trinucleotide concept as follows: the maximum of a given previous function F_k in a series of 3 successive bases and the difference between the maximum and the second highest of a given previous function F_k in a series of 3 successive bases.
Let $\tilde{t}_c$ be the c th trinucleotide in the genome sequence, i.e., constituted by the 3 bases in position i such that $c = \lceil i/3 \rceil$ (smallest integer greater or equal). Then,

$$F_k^M(c) = \max_{j=0,1,2} F_k(3c - j) \quad (5)$$

$$F_k^D(c) = F_k(3c - j_0) - F_k(3c - j_1) \quad (6)$$

so that $j_0, j_1, j_2 \in \{0,1,2\}$ are defined by the inequality $F_k(3c - j_0) \geq F_k(3c - j_1) \geq F_k(3c - j_2)$

Note: The 2 functions F_k^M and F_k^D always have values greater than or equal to 0.

The statistical significance of the 2 functions F_k^M and F_k^D is evaluated according to the parameter s based on the discrete sum (called "surface") of the values of a function in a given range R of trinucleotides. The function F_k^M (resp. F_k^D) identifies the 3 bases of the trinucleotide $\tilde{t}_c$ as coding bases if $\sum_{r \in R} F_k^M(r) > s$ (resp. $\sum_{r \in R} F_k^D(r) > s$) where R is the greatest range containing c such that $\forall r \in R$, $\max_{j=0,1,2} F_k(3r - j) = F_k(3r - j_0)$ where $j_0 \in \{0,1,2\}$ is constant. In order to visualise this concept, these 2 discrete sums $\sum_{r \in R} F_k^M(r)$ (resp. $\sum_{r \in R} F_k^D(r)$) are represented in the Fig. 1b (resp. Fig. 1c).

In summary, 8 functions are analysed with the parameter s, with F_k^M and F_k^D by varying k between 1 and 4. This statistical method is the main scientific part of the research software that is presented below.

2.3. Development of a research software called Analysis of Coding Genes (ACG)

The main functionalities of the research software called Analysis of Coding Genes (ACG) are the statistical analyses of different functions based on the circular code in sequence populations, the identification of protein genes in genomes, and the determination of their frame. Furthermore, several patterns of protein genes can be evaluated: their length, their position in the genome, their relative position between themselves, and the presence of internal frames. Several examples of these possibilities are given in the section titled Results.

The software is written with 3 units: a sequence analysis unit, a statistical function unit and an interface unit.

The sequence analysis unit reads the sequences and computes the occurrence frequency $P(T_g,w_i)$ in a window according to the algorithm described below. This unit calls the statistical function unit for computing a chosen function F_k^M and F_k^D. Precisely, the 4 functions F_k and their trinucleotide evaluation F_k^M or F_k^D are implemented in this statistical function unit, which allows statistical numerical results on a sequence population F (eventually on one sequence). The interface unit allows the choice of different statistical parameters: the EMBL sequence file (population F or sequence S), the statistical function F_k^M or F_k^D, the window length *n* in trinucleotides, and the statistical surface parameter *s*. It also has a graphical functionality for displaying the graphical curves: the start base position in the sequence, the curve display window length in bases, the left/right scroll of a curve allowing to display a curve again, and a colored curve associated with the frame for a direct interpretation. The curve display window can be printed on a broad range of printing devices. The statistical numerical results are stored in text files.

This structure in units easily allows modifications and extensions of the software ACG. ACG has been developed to be interactive and user-friendly. ACG is written in Pascal Delphi and implemented on IBM compatible microcomputers. It can be used without any computer knowledge.

The algorithm for computing the occurrence frequency $P(T_g,w_i)$ is constructed such that the different bases in each sequence are read only one time.

A window w_i of n trinucleotides runs from the first ($i=1$) base to the base position $i=l(S)-3n+1$ in the sequence. At each step of the algorithm, a base is read in the sequence and treated.

From the base position $k=1$ to $k=3n+2$, the algorithm computes the values $P(T_g,w_i)$ for $g=0,1,2$ and $i=1,2,3$.

From the base position $k=3n+3$ to $l(S)$, the algorithm computes for $i=k-3n+1$, the values $P(T_g,w_i)$ for $g=0,1,2$, i.e., from $i=4$ to $l(S)-3n+1$. The value $P(T_g,w_i)$ is deduced from $P(T_g,w_{i-3})$. Indeed, the 2 windows w_i and w_{i-3} differ only from one trinucleotide (Fig. 2). Let the "destroyed trinucleotide" t_d be the trinucleotide belonging to w_{i-3} but not to w_i, i.e., the trinucleotide from the position $i-3$ to $i-1$. Similarly, let the "constructed trinucleotide" t_c be the trinucleotide belonging to w_i but not to w_{i-3}, i.e., the last trinucleotide in w_i that starts in position $i+3(n-1)$ and ends in position $i+3n-1$ (Fig. 2). Suppose that

$t_d \in T_g$ and $t_c \in T_{g'}$. If $g \neq g'$, then $P(T_g, w_i) = P(T_g, w_{i-3}) - 1/n$ and $P(T_{g'}, w_i) = P(T_{g'}, w_{i-3}) + 1/n$. If $g = g'$, then $P(T_{g''}, w_i) = P(T_{g''}, w_{i-3})$ for $g'' = 0,1,2$.

This algorithm is implemented with 3 words indexed from 0: 2 words *d* and *c* of length 3 associated with the destroyed and constructed trinucleotides respectively, and *w* of length 3*n*, with the current window. At the step treating the *k*th base in the sequence, the base in the position *k* modulo 3*n* in the word *w* is moved in the position *k* modulo 3 in the word *d*. Then, the *k*th base in the sequence is read and placed at the position *k* modulo 3 in the word *c* and at the position *k* modulo 3*n* in the word *w*. In this way, the 3 words contain correctly the series of bases of the sequence which is read only one time.

Example of computation (Fig. 3):

The subsequence of *S* that is analysed comprises the base positions between 9 and 19. The base length of the window *w* is chosen as $3n = 6$. The proposed computation starts at the step $k = 17$, treating the 17th base in *S*. The window w_i is in position $i = k - 3n + 1 = 17 - 6 + 1 = 12$ and then contains the bases $w = \text{CCCCCC}$. The "destroyed trinucleotide" t_d is the trinucleotide starting at the position $i = 12 - 3 = 9$: the word *d* contains $d = \text{AAA}$. The "constructed trinucleotide" t_c is the trinucleotide starting at the position $i + 3(\text{n} - 1) = 12 + 3 = 15$: the word *c* contains $c = \text{CCC}$. Note that at this step $w = w_{12}$, $d = t_d$, and $c = t_c$. At the step $k = 18$, treating the 18th base in the sequence, the base in the position $(k \bmod 3n) = 0$ in the word *w* is moved in the position $(k \bmod 3) = 0$ in the word *d*. Therefore, $d = \text{CAA}$. Then, the 18th base in the sequence is read and placed at the position $(k \bmod 3) = 0$ in the word *c* and at the position $(k \bmod 3n) = 0$ in the word *w*. Therefore, $c = \text{GCC}$ and $w = \text{GCCCCC}$. Note that at this step, $w \neq w_{13}$, $d \neq t_d$, and $c \neq t_c$. The words *w*, *d*, and *c* can be obtained by circular permutations from w_i, t_d, and t_c, respectively. This method avoids base shifting in the words. This process is reiterated at the next steps (see the Fig. 3 for the step 19).

2.4. Data acquisition

The gene population *F* used for the statistical analysis is made of all human sequences (84222 sequences, 303124560 bases) obtained from release 57 (December 1998) of the EMBL Nucleotide Sequence Data Library, in the same way as described in previous studies (see e.g., Arquès and Michel, 1987, 1990 for a description of data acquisitions). This large population leads to stable frequencies for the different functions analysed (law of large numbers). Therefore, these functions can be compared in order to identify the most interesting. The protein coding genes are extracted according to the keyword CDS without discarding particular sequences. In this population, 9.4 % of bases are annotated as coding. After the validation of the statistical approach, the research software ACG has been developed for identifying protein genes and used with the human chromosomes (Sanger Centre, March 1999).

3. RESULTS

3.1. Statistical results

The different functions are evaluated with the software ACG according to the classical parameter Simple Matching Coefficient (SMC) (Burset & Guigó, 1996), which considers the proportion of bases (according to the EMBL release) identified correctly by the function. Let $\sum_{s \in F} I(S) = n_F$ be the total number of bases in the gene population *F*. Let *TP* (True Positives) (resp. *TN*, True Negatives) be the total number of bases identified as coding (resp. non-coding) bases by a function (defined above) in the coding (resp. non-coding) genes in the gene population *F*. The coefficient *SMC* is then defined as $SMC = (TP + TN)/n_F$ (Burset & Guigó, 1996).

The 8 functions F_k^M and F_k^D defined above are analysed with the coefficient *SMC*. These functions are evaluated with the parameter surface *s* between 6 and 130 with a step of 2. They are calculated with a window length *n* varying between 33 and 169 trinucleotides with a step of 17 trinucleotides. For each function, a maximum value of the coefficient *SMC* is obtained for given values of *s* and *n*. The 8 curves associated with the 8 maximum values of the 8 functions are represented in Fig. 4a,b by varying *s* for a given *n*. Fig. 4a (resp. 4b) gives the 4 curves F_k^M (resp. F_k^D).

The figures 4a,b show that the coefficient *SMC* is maximum with the function F_2^D with *n*=50 and *s*=38, which identifies 93.32 % of bases correctly.

For the function giving the maximum value of the coefficient *SMC* (F_2^D with *n*=50 and *s*=38), 4 other classical measures are computed, *Sn*, *Sp*, *Sp'*, and *CC*, as follows (Burset & Guigó, 1996). Let *FP* (False Positives) (resp. *FN*, False Negatives) be the total number of bases identified as coding (resp. non-coding) bases by a function (defined above) in the non-coding (resp. coding) genes in the gene population *F*. Note: $TP + TN + FP + FN = n_F$. The definitions and results of these 4 measures are:

(i) The Sensitivity *Sn* is the proportion of coding bases identified correctly by the function:

$$Sn = TP/(TP + FN) = 39.75\ \%$$

(ii) The Specificity *Sp* is the proportion of non-coding bases identified correctly by the function:

$$Sp = TN/(TN + FP) = 98.88\ \%$$

(iii) Another definition of the Specificity *Sp'* is the proportion of coding bases among the bases identified as coding by the function:

$$Sp' = TP/(TP + FP) = 78.71\%$$

(iv) The Correlation Coefficient *CC* is a measure of global accuracy where the value 1.00 corresponds to a perfect prediction and where the value 0.0 is expected for a random prediction:

$$CC = \frac{(TP \times TN) - (FN \times FP)}{((TP + FN)(TN + FP)(TP + FP)(TN + FN))^{1/2}} = 0.53$$

3.2. Applications of the research software Analysis of Coding Genes (ACG) with the human chromosomes

3.2.1. Three examples leading to classical results

The research software ACG identifies protein genes and their frames as follows:

- The identification of a protein gene (called CDS according to the EMBL syntax) results from a curve that is significantly greater than the 2 others that lead to a large surface *s* (notion introduced in Section Method). The existence of a top curve is justified by the fact that the associated function F_k is based on the circular code, which is a strong property of the protein genes (see Introduction). The intersection of the 2 highest curves allows for predicting a beginning and end regions of protein genes.
- The identification of a frame of a protein gene is deduced from the frame of the top curve determined from the beginning of the sequence (see the section titled Method).

Three examples of identification of protein genes (CDS) listed in the EMBL human chromosomes with the software ACG, are given.

Fig. 5a identifies a large CDS (2295 bases in the human DNA sequence from clone 512B11 on chromosome 6p24-25). Indeed, the curve becomes greater than the 2 others. Note that in a random sequence, e.g., a sequence generated with the 4 bases with equiprobability, leads to 3 similar horizontal curves. These 3 curves are gathered together before the beginning of the CDS (5' regions) and after the end of the CDS (3' regions), close to random sequences. As the top curve is C_0, the predicted frame of the CDS from the beginning of the sequence, is 0. This is in agreement with the EMBL frame (frame $(44614-1) \bmod 3 = 0$).

Fig. 5b identifies a complementary protein coding gene (cCDS in the human DNA sequence from clone E146D10 on chromosome 22) of middle size (595 bases). The cCDS chosen as an example, starts at the complementary base 17772 and ends at the complementary base 17178. As the top curve is C_0, the predicted frame of the cCDS is 0. The circular code T_0, being self-complementary, a function of F_k based on T_0, leads to the same results on the DNA complementary strand. Therefore, the association of a frame with a curve, and in particular the top curve, is identical with the CDS and the cCDS. The predicted frame 0 agrees with the EMBL frame. The determination of the frame, from the beginning of the sequence, of a cCDS starting at the complementary base position *j* and ending at the complementary base position *k* is obtained with the value *j* modulo 3. The EMBL frame with this example is then 0, as $17772 \bmod 3 = 0$.

Fig. 5c identifies a series of 5 CDS (exons in the human DNA sequence from cosmid B2046 on chromosome 6), 3 among them have a small size (about 200 bases). Their frames determined by the software ACG are 1, 2, 2, 1, and 1 respectively. All the predicted frames agree with the frames deduced from the EMBL data:

- the frame of the 1st CDS is equal to 1 as $(29039-1) \bmod 3 = 1$;
- the frame of the 2nd CDS is equal to 2 as $\left((29397 - 1) + 1 - (29245 - 1) - 1\right) \bmod 3 = 2$ (this expression is obtained from the beginning of the CDS, i.e., 29397, the frame of the previous CDS, i.e., 1, and the end of the previous CDS, i.e., 29245);

- the frame of the 3rd CDS is equal to 2 as $\left((30373 - 1) + 2 - (30228 - 1) - 1\right) \bmod 3 = 2$;

- the frame of the 4th CDS is equal to 1 $\left((30740 - 1) + 2 - (30584 - 1) - 1\right) \bmod 3 = 1$;

- the frame of the 5th CDS is equal to 1 $\left((31110 - 1) + 1 - (30953 - 1) - 1\right) \bmod 3 = 1$.

Fig. 5c gives an analysis of the relative positions of the different exons between themselves.

3.2.2. Two examples leading to unexpected results

Two unexpected results obtained in the EMBL human chromosomes with the software ACG, are given: the prediction of internal frames in the protein genes and the prediction of a coding region in the genomes.

Fig. 6a predicts internal frames in a CDS (1334 bases in the human DNA sequence from clone 1189B24 on chromosome Xq25-26.3). Indeed, there are 3 intersections of the 2 first highest curves which are associated with 4 assumed internal frames 0, 1, 0 and 2. The frameshift of 1 (resp. 2) base can be associated with 1 (resp. 2) base insertion (modulo 3) or 2 (resp. 1) base deletions (modulo 3). The internal frames can also be explained with the concatenation of coding regions whose lengths are not all multiple of 3. This CDS is mentioned as pseudogene in the EMBL file.

Figure 6b predicts a coding region in the human DNA sequence from clone 1048E9 on chromosome 22q11.2-12.2 and its frame 2. This region is associated with the primary transcript starting at 18411 and ending at 18946. Surprisingly, the predicted frame by the software ACG is equal to the EMBL frame $(18411 - 1) \bmod 3 = 2$.

4. DISCUSSION

A new statistical approach using functions based on the circular code identifies 93.32 % (coefficient *SMC*) of bases in the human sequences correctly, i.e., classifies the bases in coding and non-coding genes correctly. This approach has been evaluated with the coefficient *SMC*, which represents a good compromise between the Sensitivity *Sn* of coding bases identified correctly (39.75 %) and the Specificity *Sp* of non-coding bases identified correctly (98.88 %) (in the population studied, 90.6 % bases are non-coding). These frequencies are retrieved by varying the size of the sequence population, e.g., by eliminating the short sequences (data not shown). Indeed, a large quantity of data used for the computation leads to stable values. The parameter *s* (surface) used for evaluating the statistical significance is a concept extending the natural and simplest parameter *v* based on the value of a function for a given base position. The statistical results obtained with this parameter *v* lead to a coefficient *SMC* that is significantly low than 93.32 % (data not shown). The choice of the coefficient *SMC* for evaluating this new statistical approach in order to identify protein genes in genomes is confirmed by the Correlation Coefficient *CC* whose maximum (0.53) is also reached with the function F_2^D with *n*=50 and *s*=38. Note that if all bases are predicted as non-coding then *CC* is equal to 0.

The main purpose of this paper is to propose a completely new approach for identifying protein coding genes in genomes by using a gene model based on the circular code. Therefore, the method developed allows the global location of regions that are coding for proteins or not. The start and end of the coding

region can be predicted by the intersection of the 2 highest curves. Obviously, the exact location of the boundaries can be improved in the future by analysing in detail the start regions and the end regions of coding genes by considering, for example, the start codon ATG, the stop codons TAA, TAG, and TGA, the splicing sites, the TATA box, etc. It can also be associated with other methods for identifying protein genes in genomes, such as the codon usage methods, the methods based on the Hidden Markov Model (HMM), etc. (e.g., Shulman *et al.*, 1981, Shepherd, 1981; Staden and McLachlan, 1982; Fickett, 1982; Smith *et al.*, 1983; Blaisdell, 1983; Staden, 1984; Borodovsky and Ininch, 1993; Krogh *et al.*, 1994; Burge and Karlin, 1997; Lukashin and Borodovsky, 1998; Salzberg *et al.*, 1998; Pavy *et al.*, 1999; Shmatkov *et al.*, 1999, etc.). However, it should be stressed that the method in its actual state gives interesting results. Indeed, the Correlation Coefficient *CC* is equal to 0.53 with a data set containing 303124560 bases without discarding particular sequences. Obviously, these values become significantly better if there is a data selection before the statistical analysis, e.g., by discarding particular sequences: the sequences for which the exact location of the protein genes is determined ambiguously, the sequences encoding pseudogenes, etc. For example, the removal of pseudogenes (784 274 bases representing about 2.92 % of the gene population studied) increases the coefficient *SMC* from 93.32 % to 93,53 %.

In summary, the research software Analysis of Coding Genes using functions based on the circular code, constitutes a new approach for identifying protein genes in genomes and for determining their frame. As it is based on the circular code of protein genes of both eukaryotes and prokaryotes, it can be applied independently of the type of eukaryotic/prokaryotic organism under investigation. An example of the use of the software ACG on a prokaryotic organism (Escherichia coli) is presented in Fig. 7. Furthermore, it also allows an evaluation of the length of protein genes, their position in the genome, their relative position between themselves, and the prediction of internal frames. It can be used without prerequisite knowledge: interactivity, graphical tools, possibilities of varying the parameters (the function, the length of the window, the surface level), etc. As the user-friendly software ACG is based on a new concept (circular code), the genomes can easily be investigated for obtaining new results in this research field.

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REFERENCES

Arquès, D.G., Fallot, J.-P., Marsan, L. & Michel, C.J. (1999). An evolutionary analytical model of a complementary circular code. *BioSystems* **49**, 83-103.

Arquès, D.G., Fallot, J.-P. & Michel, C.J. (1998). An evolutionary analytical model of a complementary circular code simulating the protein coding genes, the 5' and 3' regions. *Bull. Math. Biol.* **60**, 163-194.

Arquès, D.G. & Michel, C.J. (1996). A complementary circular code in the protein coding genes. *J. Theor. Biol.* **182**, 45-58.

Arquès, D.G. & Michel, C.J. (1997a). A code in the protein coding genes. *BioSystems* **44**, 107-134.

Arquès, D.G. & Michel, C.J. (1997b). A circular code in the protein coding genes of mitochondria. *J. Theor. Biol.* **189**, 273-290.

Béal, M.-P. (1993). *Codage symbolique*. Masson.

Berstel, J. & Perrin, D. (1985). Theory of codes. Academic Press.

Blaisdell, B.E. (1983). A prevalent persistent nonrandomness that distinguishes coding and non-coding eukaryotic nuclear DNA sequences. *J. Mol. Evol.* **19**, 122-133.

Borodovsky, M. & Mc Ininch, J.D. (1993). GeneMark: parallel gene recognition for both DNA strands. *Comput. Chem.* **17**, 123-133.

Burge, C. & Karlin, S. (1997). Prediction of complete gene structures in human genomic DNA. *J. Mol. Biol.* **268**, 78-94.

Burset, M. & Guigó, R. (1996). Evaluation of gene structure prediction programs. *Genomics* **34**, 353-367.

Crick, F.H.C., Brenner, S., Klug, A. & Pieczenik, G. (1976). A speculation on the origin of protein synthesis. *Origins of Life* **7**, 389-397.

Crick, F.H.C., Griffith, J.S. & Orgel, L.E. (1957). Codes without commas. *Proc. Natl. Acad. Sci.* **43**, 416-421.

Eigen, M. & Schuster, P. (1978). The hypercycle. A principle of natural self-organization. Part C: The realistic hypercycle. *Naturwissenschaften* **65**, 341-369.

Fickett, J.W. (1982). Recognition of protein coding regions in DNA sequences. *Nuc. Acids Res.* **10**, 5303-5318.

Karlin, S., Mrazek, J. & Campbell, A.M. (1998). Codon usages in different gene classes of the *Escherichia coli* genome. *Mol. Microbiol.* **29**, 1341-1355.

Krogh, A., Mian, I.S. & Haussler, D. (1994). A hidden Markov model that finds genes in *E. coli* DNA. *Nuc. Acids Res.* **22**, 4768-4778.

Lukashin, A.V. & Borodovsky, M. (1998). GeneMark.hmm: new solutions for gene finding. *Nuc. Acids Res.* **26**, 1107-1115.

Nirenberg, M.W. & Matthaei, J.H. (1961). The dependance of cell-free protein synthesis in *E. Coli* upon naturally occurring or synthetic polyribonucleotides. *Proc. Natl. Acad. Sci.* **47**, 1588-1602.

Pavy, N., Rombauts, S., Déhais, P., Mathé, C., Ramana, D.V.V., Leroy, P. & Rouzé, P. (1999). Evaluation of gene prediction software using a genomic data set: application to *Arabidopsis thaliana* sequences. *Bioinformatics* **15**, 887-899.

Salzberg, S.L., Delcher, A.L., Kasif, S. & White, O. (1998). Microbial gene identification using interpolated Markov models. *Nuc. Acids Res.* **26**, 544-548.

Shepherd, J.C.W. (1981). Method to determine the reading frame of a protein from the purine/pyrimidine genome sequence and its possible evolutionary justification. *Proc. Natl. Acad. Sci. USA* **78**, 1596-1600.

Shulman, M.J., Steinberg, C.M. & Westmoreland, N. (1981). The coding function of nucleotide sequences can be discerned by statistical analysis. *J. Theor. Biol.* **88**, 409-420.

Shmatkov, A.M., Melikyan, A.A., Chernousko, F.L. & Borodovsky, M. (1999). Finding prokaryotic genes by the 'frame-by-frame' algorithm: targeting gene starts and overlapping genes. *Bioinformatics* **15**, 874-886.

Smith, T.F., Waterman, M.S. & Sadler, J.R. (1983). Statistical characterization of nucleic acid sequence functional domains. *Nuc. Acids Res.* **11**, 2205-2220.

Staden, R. (1984). Measurements of the effect that coding for a protein has on DNA sequence and their use for finding genes. *Nuc. Acids Res.* **12**, 551-567.

Staden, R. & McLachlan, A.D. (1982). Codon preference and its use in identifying protein coding regions in long DNA sequences. *Nuc. Acids Res.* **10**, 141-156.

T_0:	AAA	AAC	AAT	ACC	ATC	ATT	CAG	CTC	CTG	GAA	GAC	GAG	GAT	GCC	GGC	GGT	GTA	GTC	GTT	TAC	TTC	TTT
T_1:	AAG	ACA	ACG	ACT	AGC	AGG	ATA	ATG	CCA	CCC	CCG	GCG	GTG	TAG	TCA	TCC	TCG	TCT	TGC	TTA	TTG	
T_2:	AGA	AGT	CAA	CAC	CAT	CCT	CGA	CGC	CGG	CGT	CTA	CTT	GCA	GCT	GGA	GGG	TAA	TAT	TGA	TGG	TGT	

Table 1a: List per frame and in lexicographical order of the trinucleotides of the complementary circular code identified in protein coding genes of eukaryotes and prokaryotes (Arquès & Michel, 1996). Three subsets of trinucleotides can be identified: $T_0 = X_0 \bigcup \{\text{AAA,TTT}\}$ in frame 0, $T_1 = X_1 \bigcup \{\text{CCC}\}$ in frame 1 and $T_2 = X_2 \bigcup \{\text{GGG}\}$ in frame 2. The 3 sets X_0, X_1, and X_2 of 20 trinucleotides are maximal circular codes.

X_0:	AAC	AAT	ACC	ATC	ATT	CAG	CTC	CTG	GAA	GAC	GAG	GAT	GCC	GGC	GGT	GTA	GTC	GTT	TAC	TTC	
X_1:	ACA	ATA	CCA	TCA	TTA	AGC	TCC	TGC	AAG	ACG	AGG	ATG	CCG	GCG	GTG	TAG	TCG	TTG	ACT	TCT	
X_2:	CAA	TAA	CAC	CAT	TAT	GCA	CCT	GCT	AGA	CGA	GGA	TGA	CGC	CGG	TGG	AGT	CGT	TGT	CTA	CTT	

Table 1b: Circularity property with the 3 circular codes X_0, X_1, and X_2 of 20 trinucleotides identified in protein coding genes of eukaryotes and prokaryotes (Table 1a).

T_0:	AAA	AAC	AAT	ACC	ATC	CAG	CTC	GAA	GAC	GCC	GTA										
T_0:	TTT	GTT	ATT	GGT	GAT	CTG	GAG	TTC	GTC	GGC	TAC										
T_1:	AAG	ACA	ACG	ACT	AGC	AGG	ATA	ATG	CCA	CCC	CCG	GCG	GTG	TAG	TCA	TCC	TCG	TCT	TGC	TTA	TTG
T_2:	CTT	TGT	CGT	AGT	GCT	CCT	TAT	CAT	TGG	GGG	CGG	CGC	CAC	CTA	TGA	GGA	CGA	AGA	GCA	TAA	CAA

Table 1c: Complementarity property with the 3 circular codes X_0, X_1, and X_2 of 20 trinucleotides identified in protein coding genes of eukaryotes and prokaryotes (Table 1a). This property is also verified with T_0 (AAA and TTT) and T_1 and T_2 (CCC and GGG).

Codon in frame 0	Frequency (%)	Codon in frame 1	Frequency (%)	Codon in frame 2	Frequency (%)
ATG	2.31	**ATG**	3.08	ATG	0.57
CAA	1.65	CAA	1.55	**CAA**	3.71
CCA	1.66	**CCA**	2.91	CCA	2.04
GGA	1.76	GGA	1.27	**GGA**	3.49
GTC	1.60	GTC	0.81	GTC	1.05
GTT	1.55	GTT	0.75	GTT	1.35
TCC	1.63	**TCC**	1.85	TCC	1.40

Table 2: A few examples taken from the Table 1b of Arquès & Michel (1996) showing that the codons GTC and GTT belonging to X_0 occur in frame 0 with lower frequencies compared to the codons ATG belonging to X_1 and CAA belonging to X_2, etc.

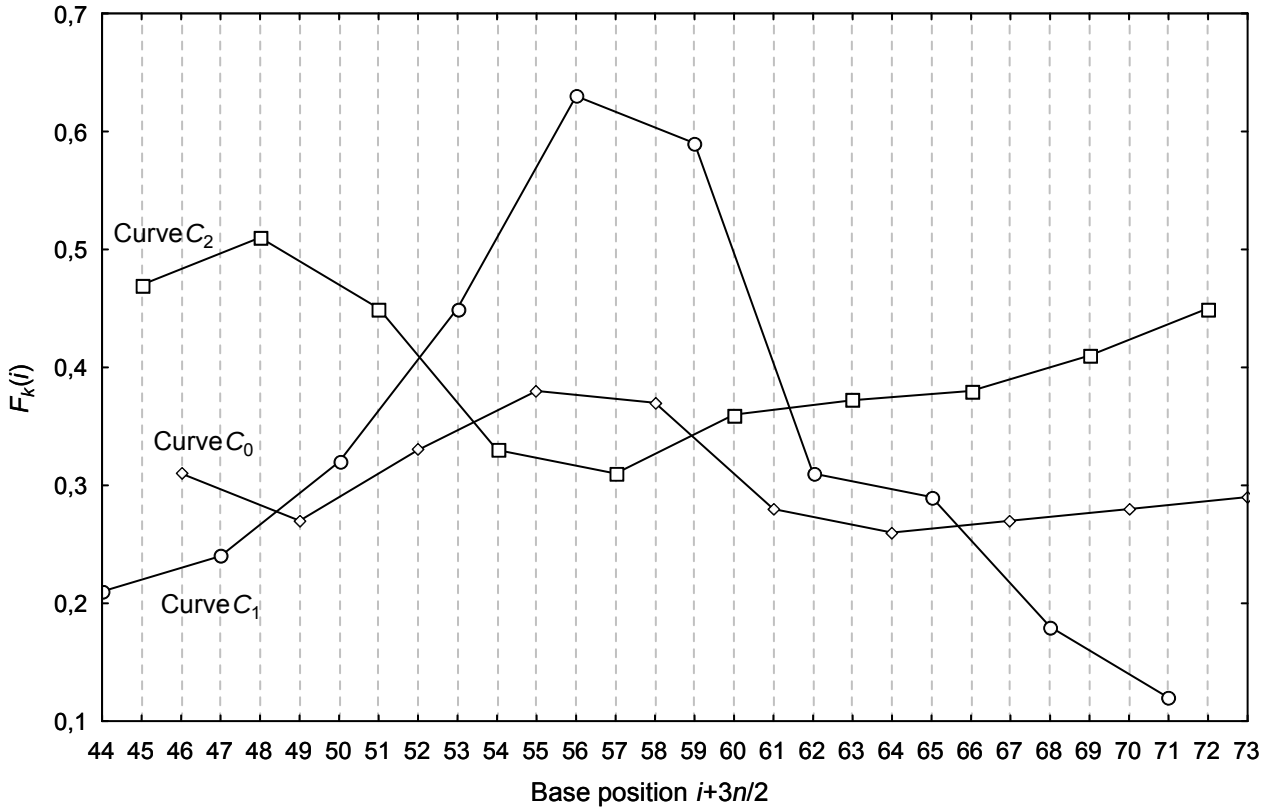

Figure 1a: Representation of a function F_k by 3 curves modulo 3. By convention, the curve C_0 (resp. C_1, C_2) joins the points in base position 1 (resp. 2, 0) modulo 3 and therefore is related to the base position in frame 0 (resp. 1, 2) determined from the beginning of the sequence.

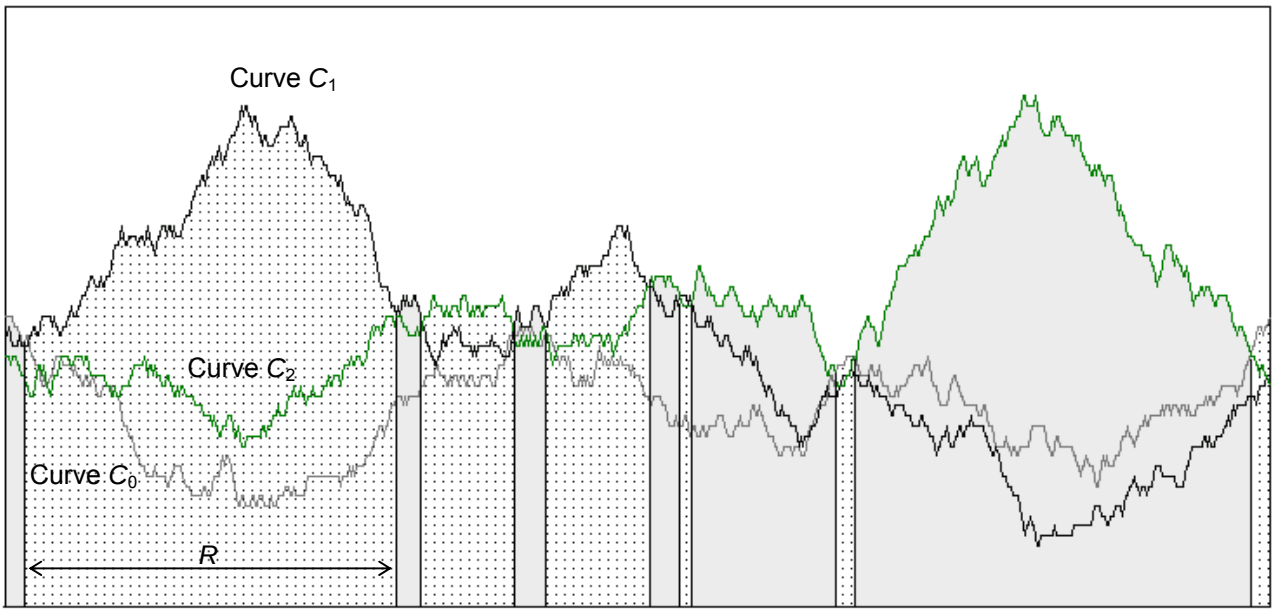

Figure 1b: Representation of the discrete sums $\sum_{c \in R} F_k^M(c)$ in different ranges R by surfaces. For a given range, the surface is associated with the highest curve.

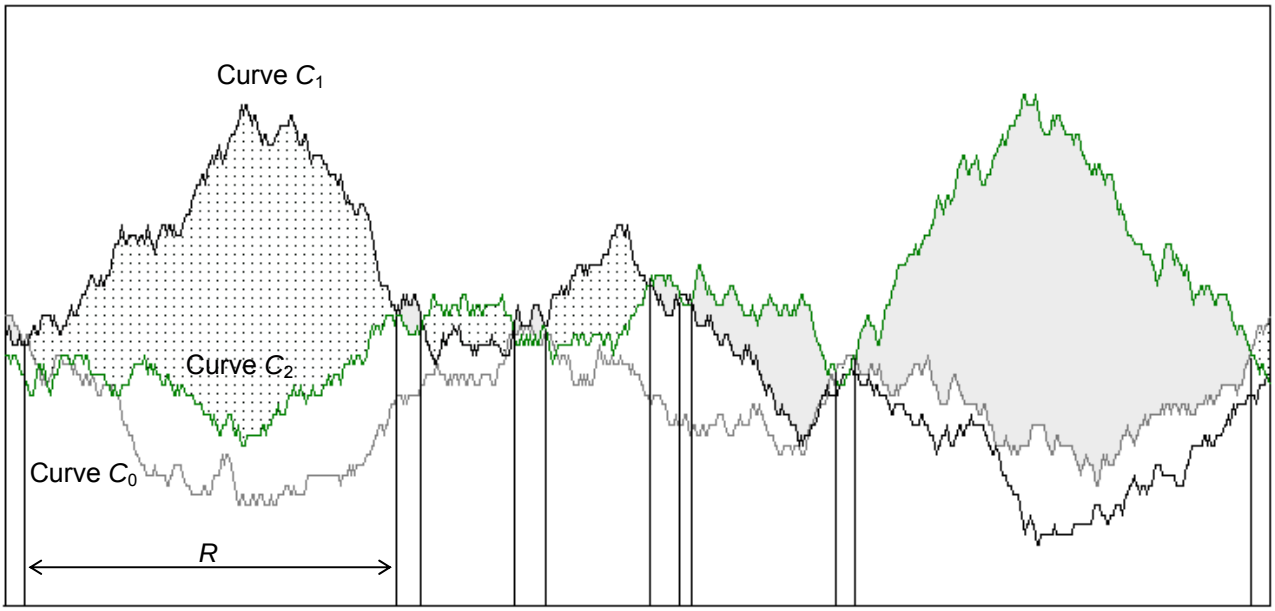

Figure 1c: Representation of the discrete sums $\sum_{c \in R} F_k^D(c)$ in different ranges R by surfaces. For a given range, the surface is associated with the difference between the 2 highest curves.

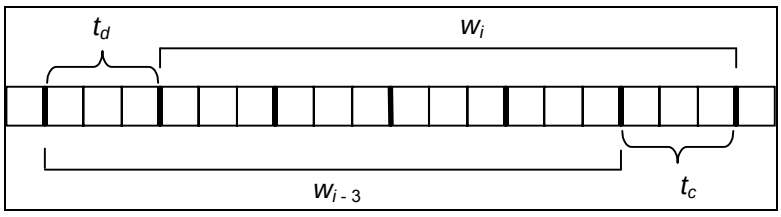

Figure 2: Computation of the 3 occurrence frequencies $P(T_g, w_i)$ in the window w_i from the window w_{i-3}. The 2 windows w_i and w_{i-3} differ only from one trinucleotide. The "destroyed trinucleotide" t_d is the trinucleotide belonging to w_{i-3} but not to w_i, i.e., the trinucleotide from the position $i-3$ to $i-1$. Similarly, the "constructed trinucleotide" t_c is the trinucleotide belonging to w_i but not to w_{i-3}, i.e., the last trinucleotide in w_i that starts in position $i+3(n-1)$ and ends in position $i+3n-1$.

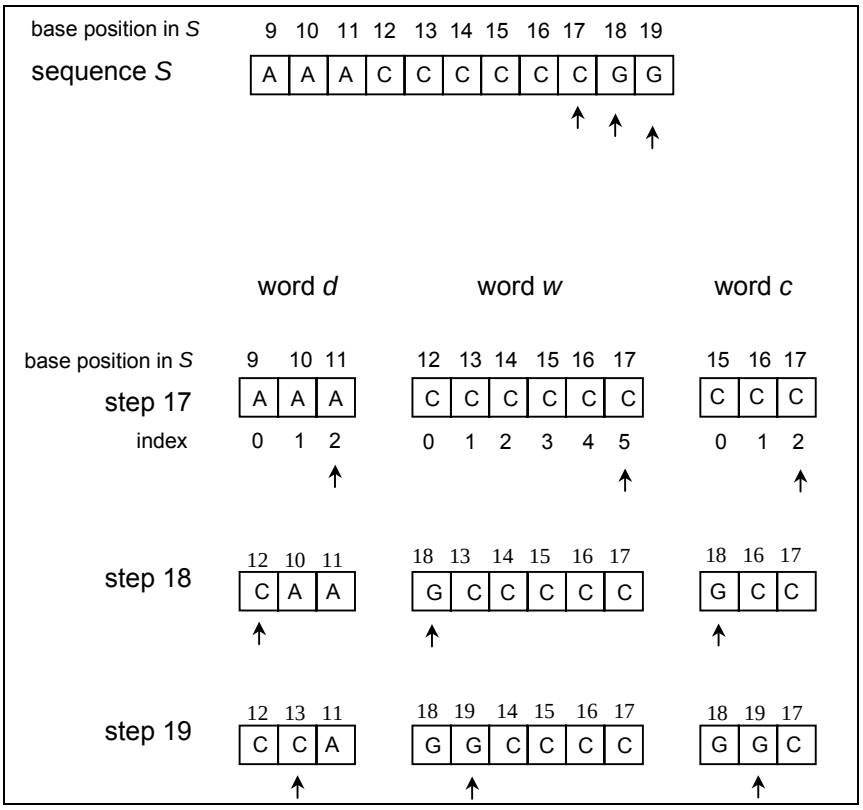

Figure 3: Example of a progress of the algorithm. This example is applied with a subsequence of *S* between the base positions 9 and 19 and with a base length of the window *w* equal to $3n = 6$. At the step $k = 17$ treating the 17th base in *S*, the window w_i is in position $i = k - 3n + 1 = 17 - 6 + 1 = 12$ and contains then the bases $w = \text{CCCCCC}$. The "destroyed trinucleotide" t_d is the trinucleotide starting at the position $i = 12 - 3 = 9$: the word *d* contains $d = \text{AAA}$. The "constructed trinucleotide" t_c is the trinucleotide starting at the position $i + 3(\text{n} - 1) = 12 + 3 = 15$: the word *c* contains $c = \text{CCC}$. At this step $w = w_{12}$, $d = t_d$ and $c = t_c$. At the step $k = 18$ treating the 18th base in the sequence, the base in the position $(k \bmod 3n) = 0$ in the word *w* is moved in the position $(k \bmod 3) = 0$ in the word *d*. Therefore, $d = \text{CAA}$. Then, the 18th base in the sequence is read and placed at the position $(k \bmod 3) = 0$ in the word *c* and at the position $(k \bmod 3n) = 0$ in the word *w*. Therefore, $c = \text{GCC}$ and $w = \text{GCCCCC}$. At this step, $w \neq w_{13}$, $d \neq t_d$ and $c \neq t_c$. The words *w*, *d* and *c* can be obtained by circular permutations from w_i, t_d and t_c respectively. This process is reiterated at the step 19.

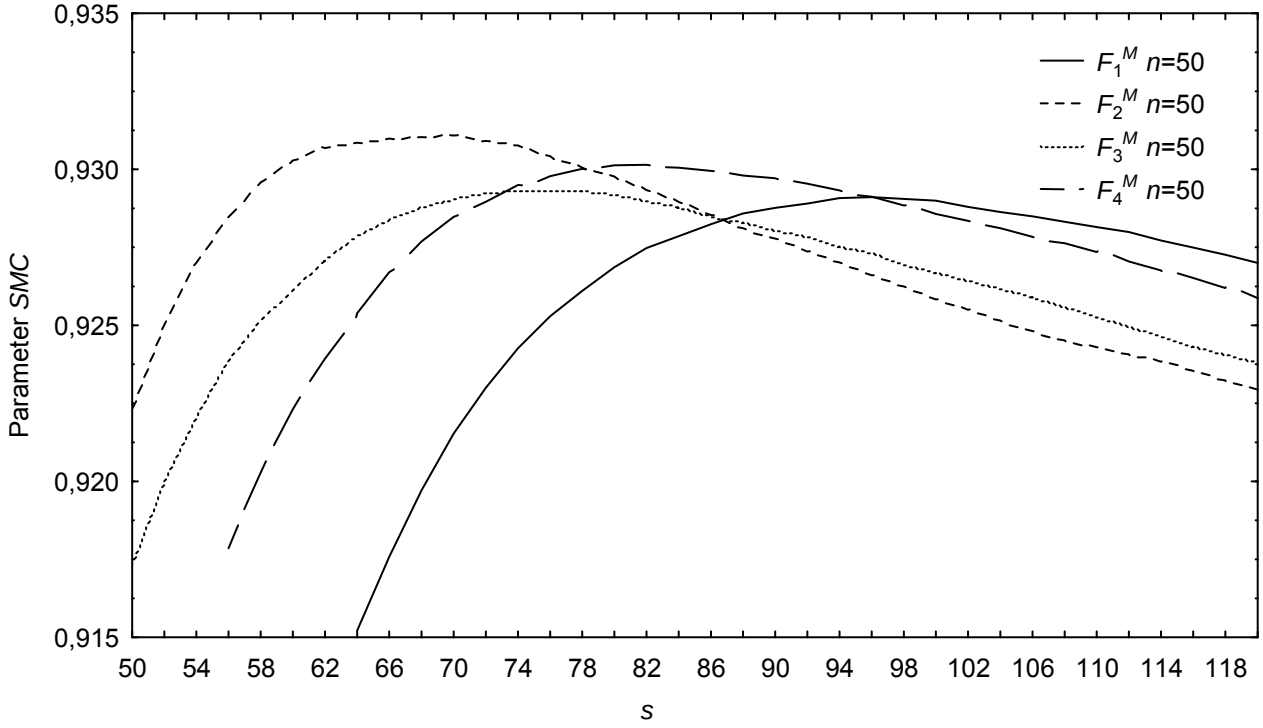

Figure 4a: Statistical results giving the proportion *SMC* of bases identified correctly by the 4 functions F_k^M. The functions F_k^M are evaluated by varying the parameter surface *s* between 6 and 130 with a step of 2 and the window length *n* between 33 and 169 trinucleotides with a step of 17 trinucleotides. The maximum value of the proportion *SMC* is given with a function F_k^M by varying *s* for a given *n*. The 4 maxima of the 4 functions F_k^M are all less than the 4 maxima of the 4 functions F_k^D (see Fig. 4b).

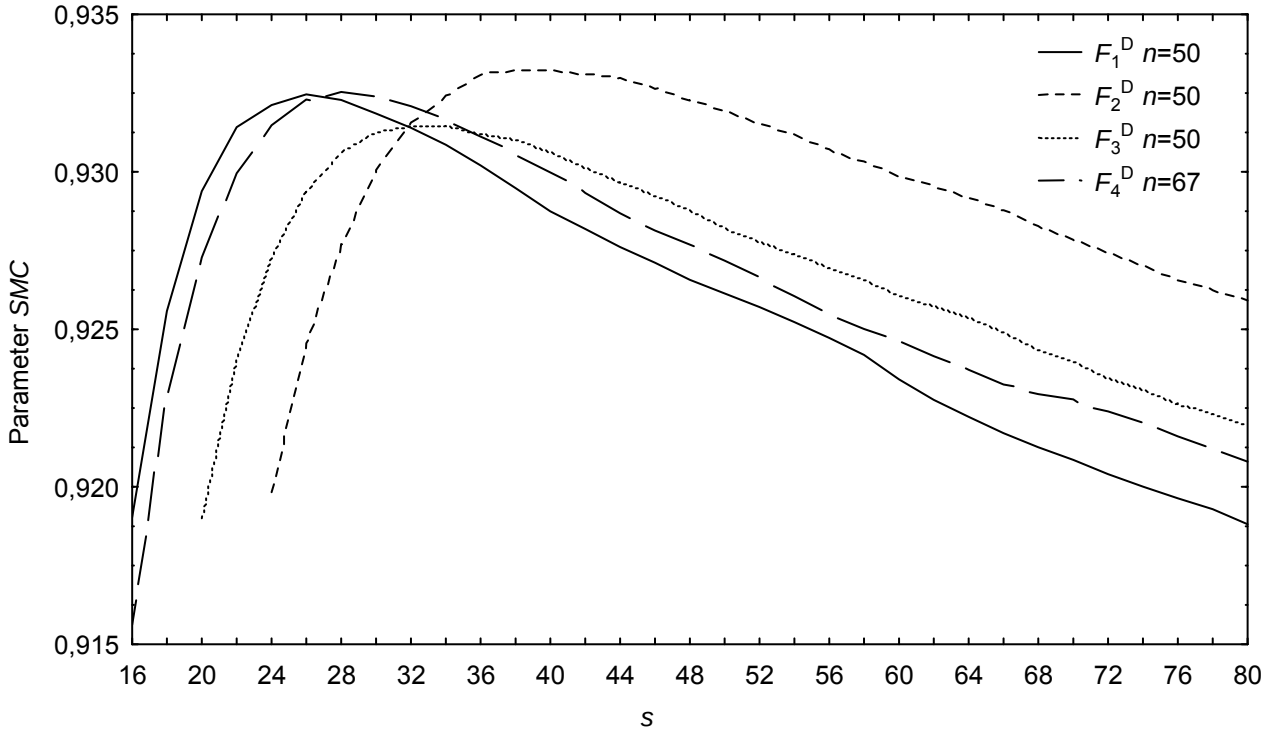

Figure 4b: Statistical results giving the proportion *SMC* of bases identified correctly by the 4 functions F_k^D evaluated by the parameter s. The functions F_k^D are evaluated by varying the parameter surface *s* between 6 and 130 with a step of 2 and the window length *n* between 33 and 169 trinucleotides with a step of 17 trinucleotides. The maximum value of the proportion *SMC* is given with a function F_k^D by varying *s* for a given *n*. The parameter *SMC* is maximum with the function F_2^D with *n*=50 and *s*=38 and equal to 93.32 % of bases identified correctly.

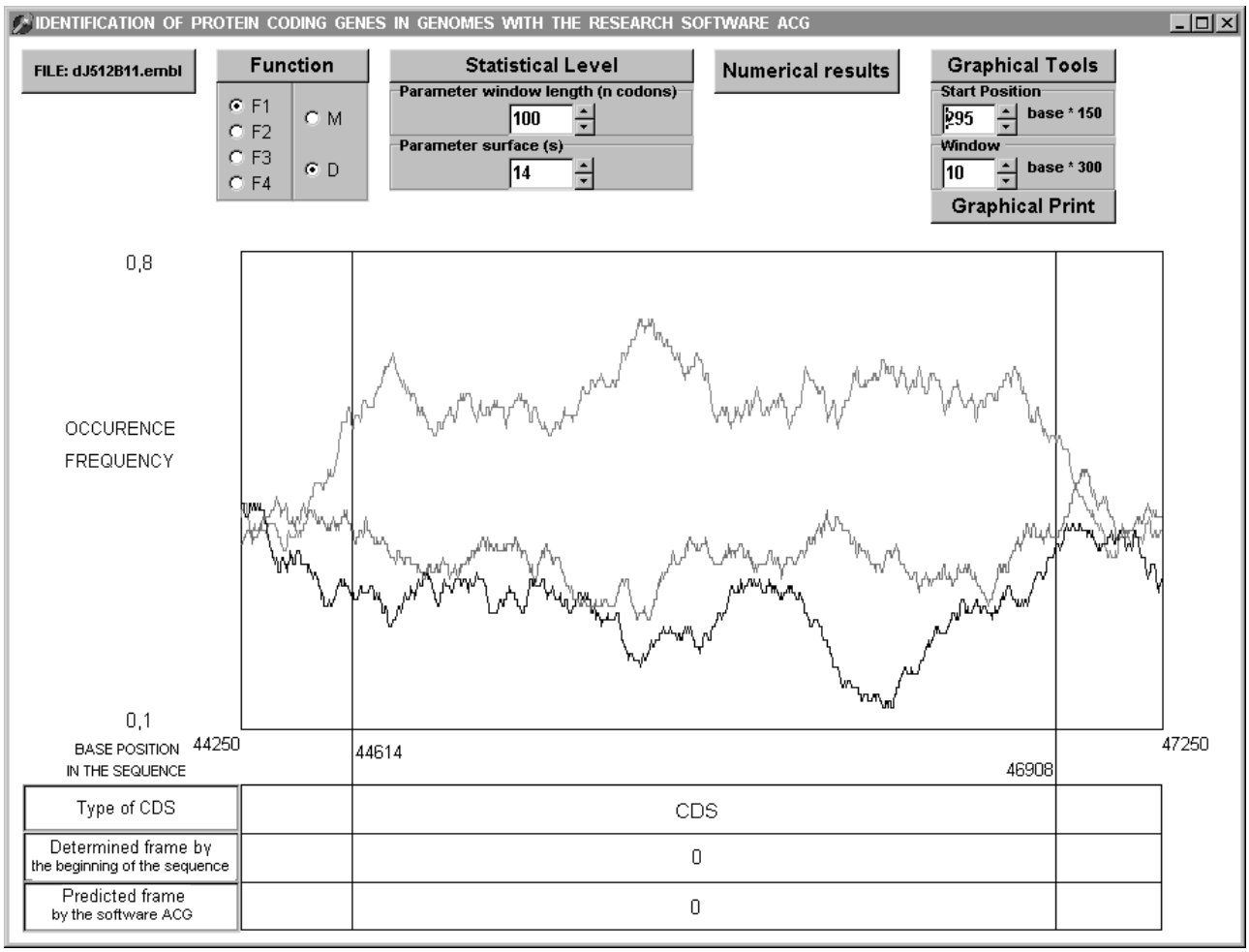

Figure 5a: Identification of a protein coding gene (CDS) and its frame by the software ACG. The CDS chosen as an example, starts at the base 44614 and ends at the base 46908 in the human DNA sequence from clone 512B11 on chromosome 6p24-25.

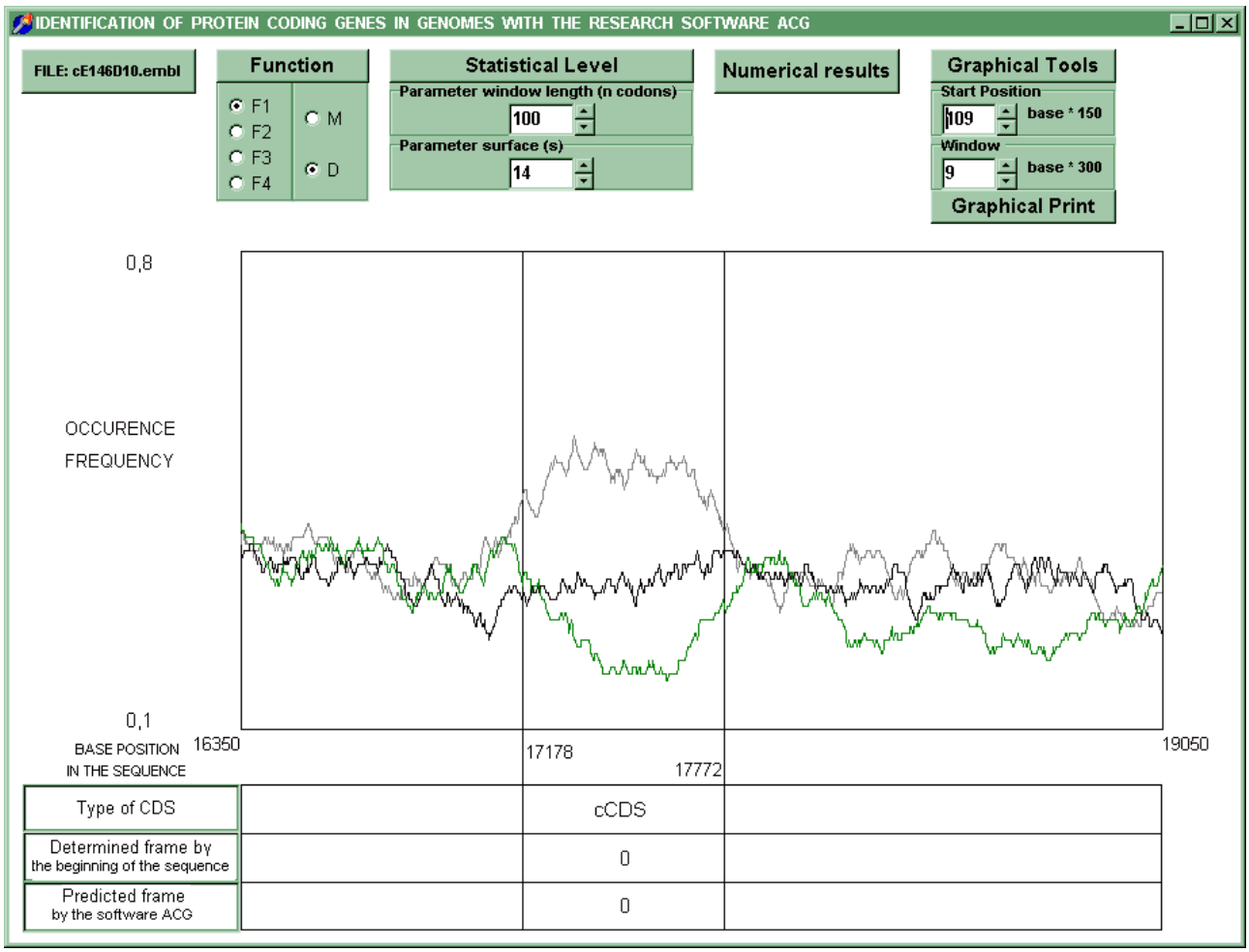

Figure 5b: Identification of a complementary protein coding gene (cCDS) and its frame by the software ACG. The cCDS chosen as an example, starts at the complementary base 17772 and ends at the complementary base 17178 in the human DNA sequence from clone E146D10 on chromosome 22.

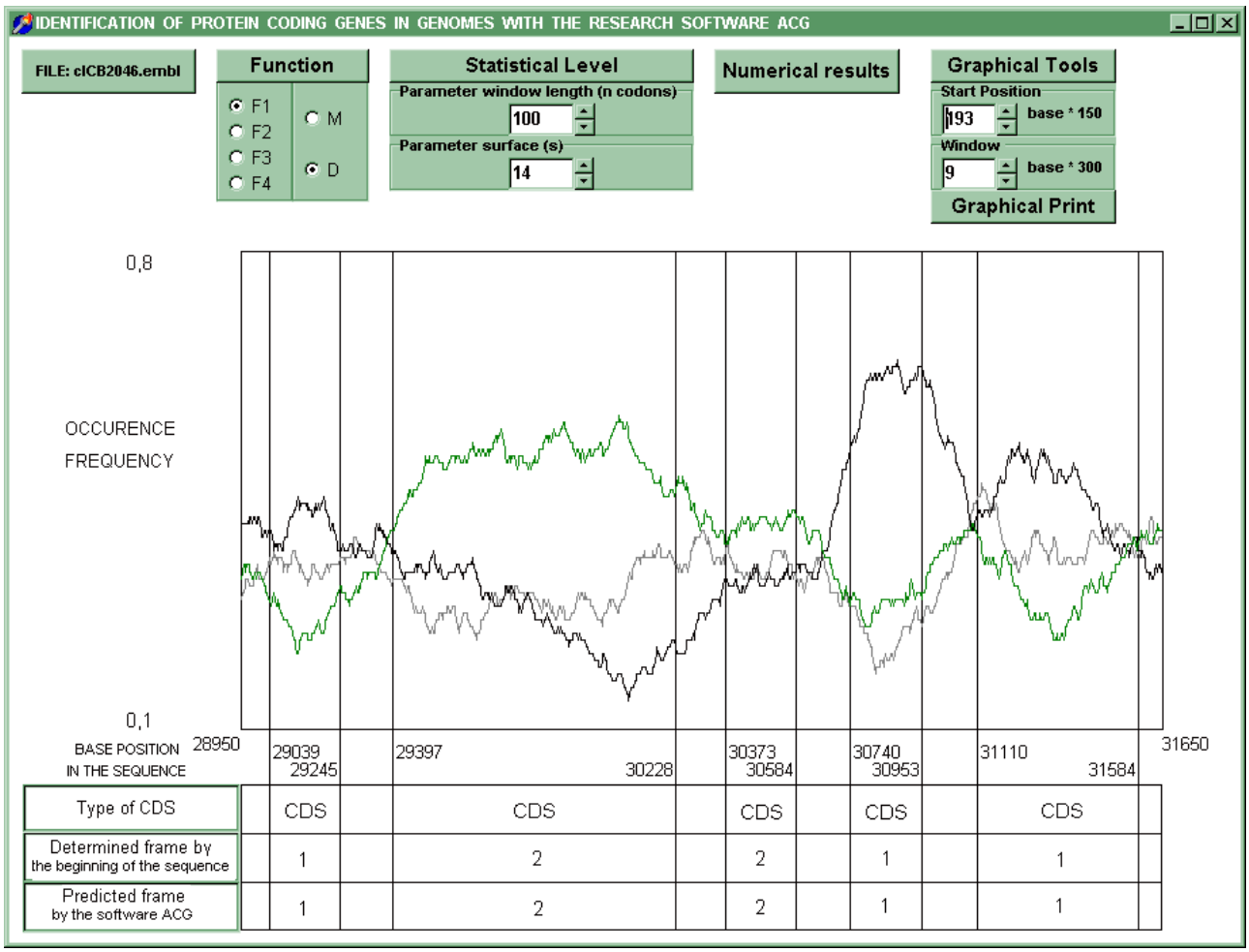

Figure 5c: Identification of several protein coding genes (CDS) and their frames by the software ACG. This example is observed in the human DNA sequence from cosmid B2046 on chromosome 6.

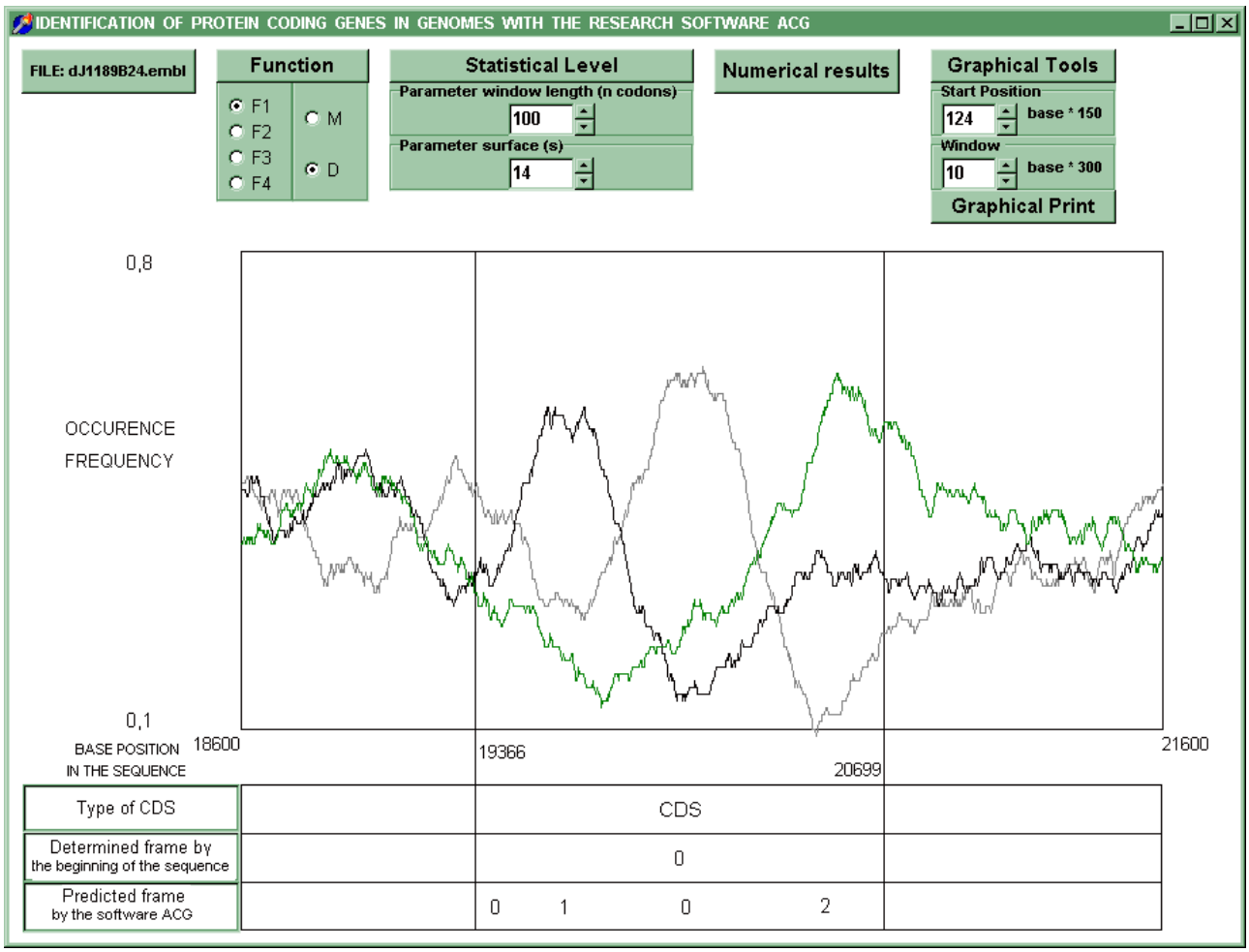

Figure 6a: Prediction of internal frames in a protein coding gene (CDS) and their internal frames by the software ACG. This example is observed in the human DNA sequence from clone 1189B24 on chromosome Xq25-26.3.

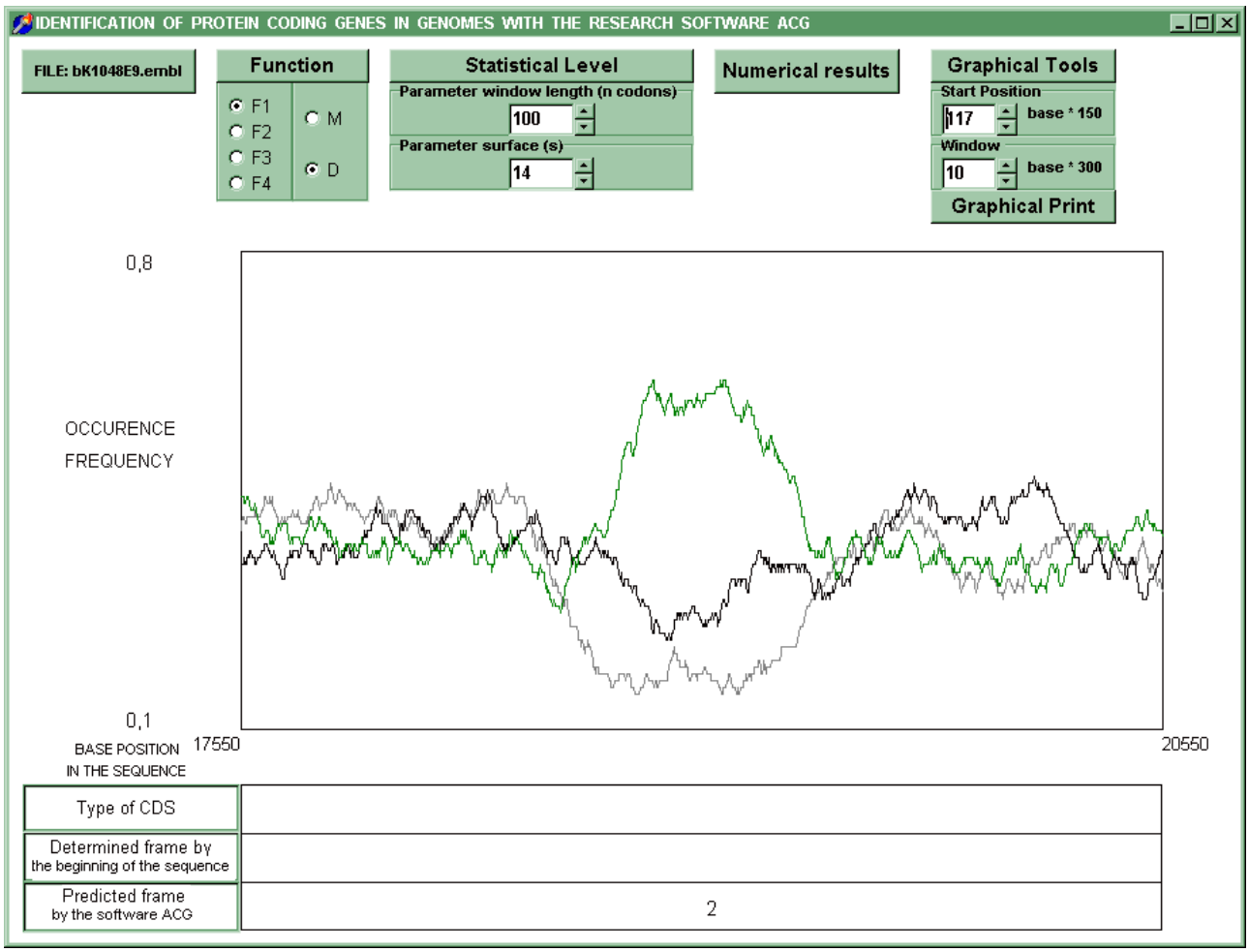

Figure 6b: Prediction of a coding region in the human DNA sequence from clone 1048E9 on chromosome 22q11.2-12.2.

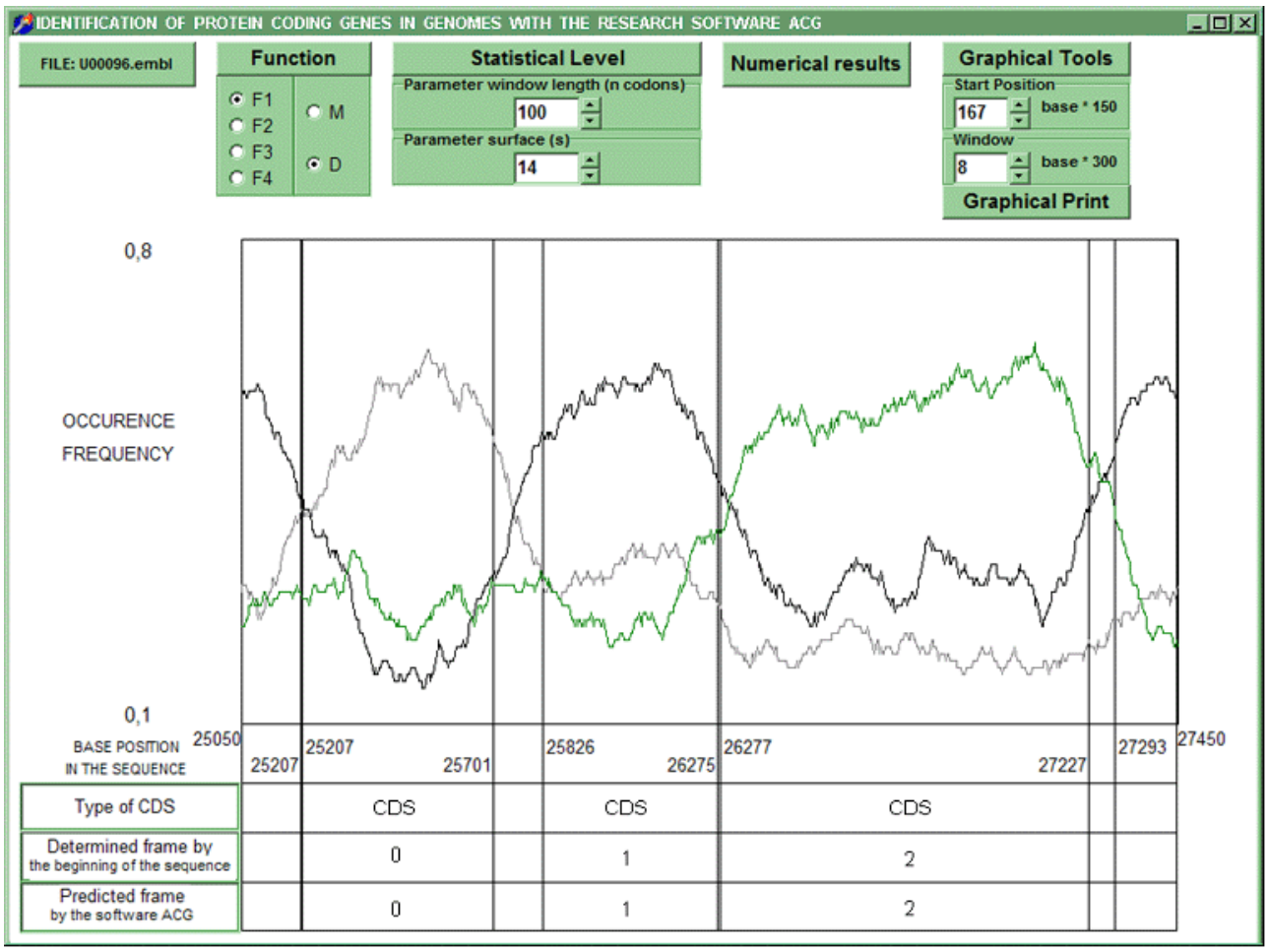

Figure 7: Identification of several protein coding genes (CDS) and their frames by the software ACG. This example is observed in the *Escherichia coli* K-12 MG1655 complete genome.