

# **DNATagger, colors for codons**

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ABSTRACT. DNATagger is a web-based tool for coloring and editing DNA, RNA and protein sequences and alignments. It is dedicated to the visualization of protein coding sequences and also protein sequence alignments to facilitate the comprehension of evolutionary processes in sequence analysis. The distinctive feature of DNATagger is the use of codons as informative units for coloring DNA and RNA sequences. The codons are colored according to their corresponding amino acids. It is the first program that colors codons in DNA sequences without being affected by "out-of-frame" gaps of alignments. It can handle single gaps and gaps inside the triplets. The program also provides the possibility to edit the alignments and change color patterns and translation tables. DNATagger is a JavaScript application, following the W3C guidelines, designed to work on standards-compliant web browsers. It therefore requires no installation and is platform independent. The web-based DNATagger is available as free and open source software at http://www.inf.ufrgs.br/~dmbasso/dnatagger/.

**Key words:** Protein-coding DNA; Codon alignment; Bioinformatics tool; Alignment colorization; Visualization and editing

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# INTRODUCTION

Genes are made of DNA (or RNA in some organisms) and carry the information for constructing whole organisms. The genetic sequences evolve over time, accumulating mutations that account for the differentiation and diversity of living beings. The comparative analysis of homologous genes provides helpful insights into the evolutionary history of genes and organisms.

Protein-coding genes are translated into amino acid polypeptides following the genetic code. The DNA sequence of a gene directly determines the sequence of amino acids in the protein it produces (Lewin, 1996). Following a determined reading frame, each group of three consecutive nucleotides in the DNA (or RNA) sequence corresponds to an amino acid residue that will be incorporated into the protein sequence. These nucleotide triplets are called "codons". The correspondence between the codons and their coded amino acids constitutes the genetic code.

With an alphabet of four letters representing nucleotides, there are 64 triplet arrangements. From these 64 codons, up to four, depending on each genetic code, are nonsense (stop codons), and they signal the end of the translation. The standard genetic code has three stop codons, while there are examples of alternative codes having one (e.g., alternative flatworm mitochondrial code), two (e.g., yeast mitochondrial code) or four stop codons (e.g., vertebrate mitochondrial code). Each of the 60 (or more) sense codons specifies one of the 20 amino acids. Consequently, each amino acid can be coded by more than one triplet. This redundancy is referred to as the "degeneracy" of the genetic code. This property allows the occurrence of silent mutations, i.e., mutations that alter the DNA sequence, while the coded protein remains with the same amino acid sequence. The information about silent mutations can be very informative in phylogenetic and evolutionary studies (Goldman and Yang, 1994). Protein-coding nucleotide sequences carry evolutionary information that is hidden in their amino acid counterparts. However, the four bases present in the DNA will not reflect the diversity of the residues in the protein (Bininda-Emonds, 2005). Thus, it is practical to combine information from amino acid and DNA sequences.

Two or more related sequences can be compared in an alignment. They are arranged in a matrix and compared in order to find similarities and differences. The sequences being compared are expected to be homologous, i.e., derived from a common ancestor. Each single sequence is placed as a row, whereas columns represent homologous sites. Over the evolutionary history, DNA sequences accumulate mutations, which can be nucleotide substitutions, insertions or deletions. To compensate insertions and deletions ("indels") in an alignment, the introduction of "gaps" is necessary (Needleman and Wunsch, 1970), normally represented by dashes. It is important to point out that, although they represent evolutionary events, gaps do not belong to the genomic sequence, and therefore, they do not interfere in the translation process.

Coloring the characters representing nucleotide or amino acid residues in multiple sequence alignments makes their visualization more agreeable and understandable. Various coloring schemes have been developed to highlight residue conservation, amino acid properties, nucleotide types, and even structural patterns (Parry-Smith et al., 1998; Beitz, 2000). Commonly used software for alignment editing, visualization and presentation, such as ClustalX (Thompson et al., 1997), CINEMA (Parry-Smith et al., 1998) and TEX-shade (Beitz, 2000), shade each character or each column in an alignment independently.

Genetics and Molecular Research 7 (3): 853-860 (2008)

Only a few programs for alignment editing and visualization consider codons and their correspondence to amino acids in order to color DNA sequences. MacClade (Maddison and Maddison, 2000) and Se-Al (Rambaut, 1996-2002) are only available for Macintosh machines. However, these programs also assume a model of evolution where nucleotide insertions and deletions always occur in multiples of three and always start and stop at codon boundaries. The requirement that indels always have to start and stop at codon boundaries does not reflect biological reality.

Guided by the need for visualization of the coding character of DNA sequences, we developed a tool for coloring nucleotide alignments on the basis of their translated sequences. DNATagger is the first program that colors codons in DNA sequences without being affected by "out-of-frame" gaps in alignments. It can handle single gaps and gaps inside the triplets. The coloring of codons allows frame-shift mutations to be easily recognized in the alignment. DNATagger is dedicated to the visualization of protein coding sequences and also protein sequence alignments to facilitate the comprehension of evolutionary processes in sequence analysis. It also provides the possibility to edit the alignments and change color patterns and translation tables (Figure 1). The simple text editing function provides freedom over the standard formats required by most applications.

The DNATagger web interface can be found at http://www.inf.ufrgs.br/~dmbasso/ dnatagger/ and it is free and open source software (FOSS).

# **MATERIAL AND METHODS**

### Software description

DNATagger is designed to colorize codons in DNA/RNA sequence alignments, and amino acids in protein sequence alignments (Figure 1). Therefore, all DNA/RNA sequences and sequence alignments are interpreted as being from protein coding genes. The coding frame is determined by the first base letter recognized in each sequence, irrespective of its position in the alignment. Gaps occurring in the sequence string are interpreted as non-informative characters, and are not included as part of the codon. For example, the sequence string ACAT--GG-C-T-A will be displayed with the colors representing the amino acids coded by the codons ACA, TGG, and CTA. The gaps will continue to be displaced in the original position, but they will not be colorized.

Sequences and alignments should be introduced as plain text on the "Edit" tab by pasting or typing. There is no need for file uploads. The user can work with his/her alignments in raw text. The simple text editing function provides freedom over the standard formats required by most applications. The sequences are interpreted with respect to the genetic code chosen by the user. There are 17 genetic codes available at the selection box on the "Translation Table" tab. By default, the standard code is used.

Amino acid and codon sequences are colored on the "View" tab following the color table selected by the user on the "Colors" tab. Several color combinations are already available. Figure 1 shows the Taylor table, constructed according to Taylor (1997). A monochromatic table was also created, motivated by the publication costs of color illustrations. The user is invited to create his/her own color table using the color bar option, or to write his/her own code. This table can be saved for future use in a text file. For reuse of this color table, the user should place this code in the code box and click on "interpret this color table code".

Genetics and Molecular Research 7 (3): 853-860 (2008)



Figure 1. Screenshots from DNATagger running on Firefox. Top left, the "Edit" tab with examples of DNA and protein alignments. Top right, the "View" tab with colored sequences. Bottom left, the "Color" tab, and bottom right, the "Translation Table" tab.

# **Implementation details**

The algorithm begins searching for codon blocks and amino acid blocks anywhere in the source text, and the colorization proceeds in accordance with the color and translation tables selected by the user. The identification of codon and amino acid blocks is made by regular expressions. An amino acid block is defined as a word boundary, followed by an amino acid letter, and then at least 3 letters from the amino acid alphabet ( $\Sigma = \{A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y\}$ ) or gaps ('-'), followed by a word boundary. A codon block is slightly more complex. It is defined as a word boundary followed by a letter from the nucleotide alphabet ( $\Sigma = \{A, C, G, T, U\}$ ), and then a nucleotide letter or gap ('-' or '.'), at least seven of any nucleotide letters, gaps or spaces, followed by a word boundary. One disadvantage is that in a few cases some words or identifiers may be wrongly understood as an alignment section. We suggest the use of identifiers containing at least one number or character that are not present in the amino acid alphabet to avoid such situations. The advantage of this method is the independence of formats, since the sequences can be arbitrarily placed in the text. It enables the colorization of most of the common alignment formats, as well as simple placed sequences.

For the colorization of codons, each triplet of nucleotide letters in the DNA or RNA sequence, beginning with the first letter of the sequence, is compared to the translation table and colored according to the amino acid it represents. Gaps or spaces inside the sequences are counted out and will not interfere in the interpretation of the coding properties. In the amino acid sequences, each amino acid letter is colored independently.

Genetics and Molecular Research 7 (3): 853-860 (2008)

The JavaScript infrastructure used in the web-based version of DNATagger improves the user's experience, providing extended functions such as color table selection and editing. It also enables both on-line and off-line execution, the former with the application being retrieved from the Internet, and the latter having the web browser load it from a local file. To create this local file, it is only necessary to save the complete web page from the on-line address. There is no need for any installation process.

The interface is coded as a regular HTML page associated with a CSS (Cascading Style Sheet, a W3C standard technology), with several elements connected to the program's core using event handlers. The program is composed of four objects, each encapsulating the methods to deal with menu selection, color table editing and selection, translation table selection, translation, and alignment colorization. The communication between the interface and the application's objects is shown in Figure 2. Almost all interactions back to the user are made through the manipulation of the DOM (Document Object Model) tree.

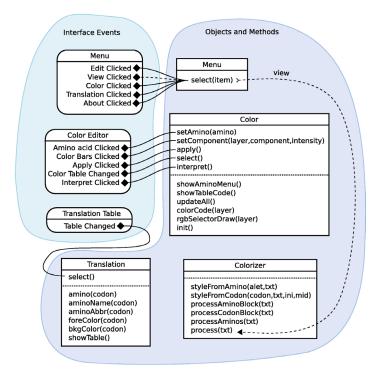


Figure 2. Communication between the interface and the application's objects.

The first version of DNATagger was written in C++ and designed as a command line application. It accepts strictly Clustal/aln format for the input file, with restriction on the lengths of the sequence names. The output is an HTML file with the colored sequences also in Clustal/ aln format. The advantage of this method is the possibility to integrate it as part of a script. The disadvantage of the first version is the format restriction. The sequence names must have exactly six characters and must be followed by exactly 10 blank spaces. We are now improving

Genetics and Molecular Research 7 (3): 853-860 (2008)

this script to allow other formats, as in the web version. It will soon be available on request.

# **RESULTS AND DISCUSSION**

## Comparison with other tools

In recent years, diverse programs, web servers and web-based applications have been improved to provide researchers in molecular biology and related areas with powerful tools for the analysis of molecular data. DNATagger is a helpful application to visualize and improve the results of coding DNA/RNA and peptide alignments. The differential feature of this program is its interpretation of nucleotide sequences as protein coding, i.e., triplets of bases as codons. Particularly important is its capability to work with "out-of-frame" gaps, interpreting each sequence in the alignment independently. Other programs that offer the possibility of coloring codons with respect to the coded amino acid cannot handle gaps that occur inside codon boundaries. The proprietary software MacClade (Maddison and Maddison, 2000) and the freely distributed software Se-AI (Rambaut, 1996-2002) have this property. Both offer a data editor tool with an option for proteincoding data that uses the position of the nucleotides in the alignment to determine the reading frame. Gaps inside the codons are treated as ambiguous characters that are part of the codon itself. This causes an artificial shift in the reading frame, and the rest of the sequence is misinterpreted until a complementary number of gaps compensate the error (Figure 3).

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igPFR3   A T6 60T 61T TTC T6C TRC 6R5 6RT 6RC 6RC C + C C CTT CAT TR 1CC TCC 60C TA - 60C TOT TCA R6T CCT TTG TCC TRA 8T6 CCB AC - ARC CTC     ig45E7   A T6 60T 61T 6TC R6T TAT 0AG TTT 0AG 6TA ACC - TC CCC AAT TGC TCC 68C TA - 60C TTT TCA R6T CTT TTG TT TTG 7C TRA 8T6 CCT CC - ARC ATT     ig45E7   A T6 60T 61T 6TG R6T TAT 0AG TTT 0AG 6TA ACC - TC CCC AAT TGC TCC 68C CA - 60C TTT TCA R6T CTT TTG TTG 7C TRA 8T6 CCT CC - ARC ATT     ig45E7   A T6 60T 61T 61T 6AT TAT 0AG TTT 0AG 6TA ACC - TC CCC AAT TGC TCC 68C CA - 60C TTT TCA R6T CTT TG TTG 7C 10A 80C CT CC CC ARG ATT     ig45E9   A T6 60T 61T 61T 61T 1T 0AG 6TA ACC - TC CCC AAT TGC TCC CCC TAC 6AT TGC TCC TTG TA ATG CTT TTG TTT TTG 7TC TTG 7AG CCC ARG CTT     ig479.1   A T6 60T 61T TT CAAT TRC GAR RCT GAR 6TT CCC TCC TAC CCC TGC TA - 60T TGT TCA ATG CTC TTG ARG CTG CCC ARG CTT     ig479.1   A T6 60T 61T TTC ART TGC GAR TCC GAR CCC - TC CCT CCC TAC CTA AGT GTT TGC TCT TG ARG CTG TTA 65 GT 6C CT TG AGT GTT TCC TTG ARG CTG CCC TG TA - 60T TGT TCA ATG CTG CTG TA 60T GTT TCC TTG ARG CTG CCC ARG CTC	#FPK3   AT6 BOT GTT TTC TGC TAC GAG GAT GAG GCC ACC - TC COT TAT CCC TCC GGC TA - GAC TGT TCA AGT CCT TTG TCC TAG GTC ACC - ARC CTC ATT     Y45E7   AT6 GGT GTT GTG AGT TAT GAG TTT GAG GTA TGC TCC GAC TA - GGC TTT TCA AGG CTT TTG TGT TG GGC GCC - ARG ATT TGC     YC94   AT6 GGT GTT GTG AGT TAT GAG TTT GAG GTA ACC - TC CCC AAT TGC TCC AGC CA - GGC TTT TCA AGG CTT TTG TGT TG GGC GTG CC - ARG ATT TGC     YC94   AT6 GGT GTT GTG AGT TAT GAG TTT GAG GTA CC - TC CCC AAT TGC TCC AGC CA - GGC TTT TCA AGG CTT TTG TTC TG GGC GTG CC - ARG ATT TGC     YC94   AT6 GGT GTT GTG AGT TAT GAG TTG GGC GTA CC - TC CCC AAT TGC TCC AGC CA - GGC TTT TCA AGG CTT TTC TTG TGC TGC CC AGG GTA TGC TCC AGC     Y6485   AT6 GGT GTT TTG GAT TAT GAC TTG GAG GTA ACC CTC CCC CC GC TA TGC TCC AGC CTA TGC TTG TTG TTG TTG TTG TTG TTG AGC CTG CCC AGG GTA TGC TGC AGG GTT TTC AGG CTT TTG AGC CTG CC AGG GTA TGC TGC AGC CTG TTG TGC TTG AGG GCT TTG TTG AGG CTG CCC AGG GTA AGC CTG CC AGG GTA TGC CCC TGC TA - GGT TGT TCA ATG CCC TTG TTG AGG GTA CCC AGG GTA AGC CTG TC AGG AGC CTG TGC AGG AGG AGC AGC CCC AGG GTA AGC CTG TGC AGG AGC AGC CCC TGC TA - GGT TGT TCA ATG CCC TTG TTG AGG GTA CCC CTG AGG AGC AGC CCC TGC TA - GGT TGT TCA ATG CCC TTG TTG AGG AGC AGC CCC TGC AGG AGC AGC CCC TGC TA - GGT GT TCA AGG CCT TTG AGG AGC AGC CCC AGG AGC AGC CCC TGC TA - GGT GT TCA AGG CCT TTG AGG AGC AGC CCC AGG AGC AGC CCC TGC TA - GGT GT TCA AGG CCT TTG AGG AGC AGC CCC AGG AGC AGC CCC TGC TA - GGT GT TCA AGG CCT CTTG AGG AGC AGC CCC AGG AGC AGC CCC TGC TA - GGT TGT TCA ATG CCT CTG TG AGG AGC AGC CCC AGC AGC CCC TGC TA - GGT TGT TCA ATG CCT CTG CTG AGG AGC AGC CCC AGG AGC AGC CCC AGC AG	9FPK4		ATG GGT	GTT TT	C AGC TA	CGAG	GAT G	AG GC	CACC	-TC	ССТ	ат С	сст	00 000	TA-	GGC	төтт	CRA	эт со	ттт	бТС	ста	GATO	CCG	AC-	AAC C	тсатт
94557 ATG GGT GTT GTG AGT TAT GAG TTT GAG GTA ACC TC CCC ART TGC CAGC CA- GGC TTT TCA AGG CTT TTG TTC TTG AGG CTC C- ARG ATT   50557 ATG GGT GTT TG GGG AGT TAT GAG TTT GAG GTA ACC TC CCC ART TGC TCA GGC CA- GGC TTT TCA AGG CTT TTG TTC TTG AGG CTC C- ARG ATT   50295 ATG GGT GTT GTG ABT TAT GAG TTT GAG GTA ACC TC CCC ART TGC TCA GGC CAG GGC TTT TCA AGG CTT TTG TTC TTG AGG CTG CC- ARG ATT   50295 ATG GGT GTT GTG ABT TAT GAG TTG GAG GTA ACC CCC CCC CAG CAG CAG GGC TTT CA AGG CTT TTG TTC TTG ARG CTG CCC ARG ATT   50495 ATG GGT GTT TG AGT CAGC TCA GCC GC CTC CCC CAG CCAA GGC CTA AGG CTT TTG TTC TTG AGG CTG CCC ARG CTA CCC CC CTC CCC CAG GTT TTC AGG CTG CTG CAGC CAA GCC TTT TG TTC TTG TTG TTG TTG TTG TTG	P445E7   ATG BOTGTT GTG ABT TAT GAG TIT GAG GTA ACC - TC CCC ART TGC TCC AGC CA- GGC TIT TCA AGG CTT TTG TCT TG BGG CTG CC- ARG ATT GC     XXC94   ATG GGT GTT GTG ABGT TAT GAG TIT GAG GTA ACC - TC CCC ART TGC TCC AGC CA- GGC TIT TCA AGG CTT TTC TTG TCT TG BGG CTG CC- ARG ATT TGC     XXC94   ATG GGT GTT GTG ABGT TAT GAG TIT GAG GTA ACC - TC CCC ART TGC TCC AGC CA- GGC TIT TCA AGG CTT TTC TTG TG GC GTA CC - AGG ATT TGC     XXC94   ATG GGT GTT GTG ABGT TAT GAG TTT GGAG GTA ACC CTC CC ART TGC TCC AGC CA- GGC TIT TCA AGG CTT TTC TTG TG GC GTA CC CC CC AGG ATT TGC TCC AGG CTT TG TTC TG TTC TTG TTG TTG TG TG TG TG TG TG TG TG TG			ATG <mark>GGT</mark>	GTT <mark>TT</mark>	с тос та	C GAG I	GAT G	AG GC	сасс	-тс	ССТ	гат с	сс ті	c ee	та-	GGC	төтт	ся в	эт со	ст тт	в то	с тя	с атс	CCG	вс-	аас с	тсятт
50,485 АТО ВОТ ОТ ТОТО НА ТТА О НО ТТО О НО ОТА АСС СТО ССО НА ТОСТСС АВС СНАВОС ТТТ ТСА ВОВ СТТ ТТО ТТО ТТО НА СТО ССО НАВОТ Т 135,49 АТО ВОТ ОТТ ТТО АПТ ТАС ОНА АСТ ОНО ТТС АСС ТТО СОТСАТТСС ССО САВТОВСТТОТ ТСА АТО ССТ ТТО ТТО ТТО АТО ССО АТО ВОСТОТ ТТО КАВТ ТАС ОНА ТСТ ОНО ТСА СС АСС СТО СОТСАТТСС СССТОС ТА ОВТТОТ ТСА АТО ССТ ТТО ТТО ТТО АТО СО ВС	400 485 ATG BOT GTT GTG AGT TAT GAC TTG GAG GTA ACC CTC CCC AAT TGC TCC AGC CAA BOC TTT TC A AGG CTT TTG TTC TGA AGC CTG CCC AAG GTT TGC TCC AGG CTG TTG TCC AGG CTT TTG ATG CTG CCC AAG GTT TGC TCC AGG CTG TTG TCC AGG CTT TTG ATG CTG ATG CTG AGG CTT TTG ATG CTG ATG CTG AGG CTG CTG CTG AGG CTG CTG CTG AGG CTG CTG CTG AGG CTG CTG CTG AGG CTG CTG CTG AGG CTG CTG AGG CTG CTG AGG CTG CTG CTG CTG AGG CTG CTG CTG CTG CTG CTG CTG CTG CTG C			ATG <mark>GGT</mark>	бтт <mark>бт</mark>	<mark>а аст та</mark>	T GAG	ттт б	AG GT	а асс	-тс	CCC	ал т	GC TI	C AG	CA-	GGC	TTT	CA 🖪	66 CT	гт тт	бтт	с тт	G <mark>AGC</mark>	стб	cc-	AAG A	TT TGG
43549 AT 6 <mark>66T GTT TTC AAT TAC GAA ACT GAG TTC ACC</mark> - TC CGT CAT TCC CCC TGC TA - <mark>66T TGT TCA AT6</mark> CCT TTG TTC TTG AT6 CTG AC - AAC CTC I AVPJ1 AT 6 <mark>66T GTT TTC ACA TAC GAA TCT GAG TCC ACC</mark> - TC CGT CAT CCC CCC TGC TA - <mark>66T TGT TCA AT6</mark> CCG CTC TTG AT6 GTG AC - <mark>AAA</mark> CTC I	43549 А ПО ВОТ ОТ ТТС АРАТАССАРА <mark>НОТ</mark> ОВОТТО АСС ЭТС БОТ СРАТИССССТОС ТА-ВОТТОТТО А ПО ВОТТИ ТТС АТО СТА ВОТОВОТ И РЈІ АТО ВОТ ОТ ТТС АСА ТАС ОВА ТСТ ОВО ТСС АСС - ТС СОТ САТ СССССТОС ТА-ВОТТОТТСА АТО БОЛ СТО СТС ТТС АТО СОТ У 44.07 А ТО ВОТ ОТ СТТС АСАТАС САРА ТСС ОВА ТС С АТО ССССТОС ТА-ВОТТОТТСА АТО БОЛ СТО СТС АТО ВОТ ОТ СТО АТО ВО			ATG <mark>GGT</mark>	GTT <mark>GC</mark>	G AGT TA	TGAG	ттт б	AGGT	а всс	с-тс	ccc	ант т	вс ті	C AG	CA-	GGC	ттт 1	ся я	66 C1	гт тт	бтт	стт	G AGO	стб	cc-	AAG A	тт төс
3549 A TO GOT GTT TTO AAT THO GAA ACT GAG TTO ACC -TO COT CAT TOC COO TGO TAT TOA ATO COT TTO TTO TTO TTO TAG ATO COO ACCARD ON A WPJ1 A TO GOT GTT TTO ACA THO GAA TOT GAG TOC ACC - TO COT CAT COO COO TAT GOT TGT TOA ATO COA TG TO TTO ATO GTG AC - AAA CTO I	35 3-9 А ГОВОТСТТТС АЛТ ТАС ОАЛ АСТ ОА ВТО А СС - ТО СОГТОЯТ СО СОСТОСТА - ОСТТОТТОА А ГОВОТСТТТС А ТО СОА СА - ИРЈІ А ГОВОТСТТТС А СА ТАС ОАЛ ТСТОА ВТОСА СТ СОСТОАТ СО СОСТОСТА - ОСТТОТТСА А ГОВОТСТО СТОАТОВ СО А СА - А АЛ «14 А ГОВОТСТТС СА ТАС ОАЛ ТСС ОАЛ ТСО СА АТТО А СС - ТО СОСТОСТА СОСТОСТА - ОСТТОТТСА А ГОВОТСТТСТО А ТО СА БО			ATG <mark>GGT</mark>	өтт өт	саст та	TGAC	TTG G	AGGT	а ясс	стс	ccc	ант т	GC T	C AG	сяя	GGC	ттт т	ся н	36 C'	гт тт	бтт	стт	G 880	стб	CCC	AAG G	тт төс
WPJ1 AT 5 05T GTT TTC ACA TAC GAA TCT GAG TCC ACC - TC CGT CAT CCC CCC TGC TA - GGT TGT TCA ATS CCA CTS CTC TTS ATS GTG AC - AAA	NPJ1 AT 6 GOT GTT TTC ACH TAC GAA TCT GAG TCC ACC - TC CGT CAT CCC CCC TGC TA - GOT TGT TCA ATG CGA CTG CTC TTG ATG GTG AC - AAA CTC ATG 34LA7 ATG GGT GTC TTC ACA TAC GAA TCC GAA TTC ACC - TC CGT CAT CCC CCC TGC TA - GGT TGT TCA ATG CCT TTG TTC TTG ATG CTG ACC ATC			ATG <mark>GGT</mark>	GTT <mark>TT</mark>	с аат та	CGAA	аст б	AG TT	C ACC	-тс	CGTO	ат т	CC CI	ос та	TA-	GGT	төт 1	сяя	T G <mark>C (</mark>	ст тт	бтт	стт	GATO	вств	AC-	AACC	тсатс
34LA 7 AT 6 GOT GTC TTC ACA TAC GAA TAC GAA TTC ACC - TC COT CAT CCC CCC TOC TA- GOT TOT TCA ATG CCT TTG TTC TTG ATG CTG AC- AAC CTC				ATG <mark>GGT</mark>	GTT <mark>TT</mark>	с аса та	C GAA	тст в	AG TO	сясс	-тс	сөто	ат с	cc ci	с та	TA-	GGT	төтт	CA A	T G 🖸	<mark>ЗА</mark> СТ	бст	стт	GATO	GTG	AC-	AAA C	тсятс
	WPI3 AT 6 SOT OTT TTC ACA TAC GAR TCC GAG TTC ACC - TO TAT CAT CCCCCCC TOC TA - GOT TOT TCA ATG CCT TTG TTC TTG ATG CTD AC - AAC CTC ATG	B4LA7		ATG <mark>GGT</mark>	өтс <mark>тт</mark>	с аса та	C GAA	тос в	AA TT	C ACC	<mark>с-тс</mark>	сөто	ат с	cc ci	с тө	та-	GGT	төт 1	CA A	T G <mark>C (</mark>	TTT	GTT	стт	бато	в ств	AC-	AAC C	тсятс
4VPI3 AT 6 GGT GTT TTC ACA TAC GAA TCC GAG TTC ACC - TC TAT CAT CCC CCC TGC TA - GGT TGT TCA AT6 CCT TTG TTC TTG AT6 CT6 AC - AAC CTC I		4VP13		ато <mark>вот</mark>	GTT <mark>TT</mark>	с аса та	C GAA	тос в	AG TT	IC ACC	о-тс	TAT	ат с	cc ci	с та	та-	GGT	төт т	CA A	га са	от тт	бтт	стт	бато	сто	AC-	аас с	тсатс
			1.	40																								

Figure 3. Partial alignment of PR-10 sequences. A. Displayed by MacClade version 4.0 PPC (Maddison and Maddison, 2000). B. Displayed by Se-Al v2.0a11 Carbon (Rambaut, 1996-2002).

Genetics and Molecular Research 7 (3): 853-860 (2008)

#### DNATagger, colors for codons

Manual comparisons between nucleotide- and amino acid-based alignments help in the identification of frame-shift mutations in the sequences. Some frame-shift mutations are very clear. The example of the partial alignment of PR-10 (Pathogenesis-related Protein 10, data from Scherer NM, unpublished results) sequences in Figure 4 shows a balanced frameshift mutation that can only be recognized in the DNA alignment. The most often used approach to align nucleotide sequences of protein coding genes is the "back-translation" of amino acid alignments (Wernersson and Pedersen, 2003). Comparing the nucleotide alignment (Figure 4A) with the alignment of the translated sequences (Figure 4B), it is clear why the amino acid alignment will not reflect the real evolutionary history of these sequences by the use of a "back-translation" approach. The reason is that the codon positions as triplets in the amino acid-based alignment are no longer really homologous. In a study of molecular evolution based on codon models (for example, Goldman and Yang, 1994), the inclusion of these "artificially aligned" codons will not represent the actual history of the site, and is therefore not compatible with the analysis. In this case, it is recommended to exclude that sequence or this excerpt of the alignment from the analysis.

#### Edit View Colors Translation Table Instructions About DNATagger

A. DNA alignment Q9FPK4 Q9FPK3 0945E7 Q6XC94 0604B5 Q43549 04VPJ1 Q84LA7 Q4VPI3 B. Back-translation from protein alignment Q9FPK4 09FPK3 Q945E7 06XC94 Q6Q4B5 Q43549 04VPJ1 Q84LA7 04VPI3

**Figure 4.** Partial alignment of PR-10 sequences displayed by DNATagger. **A.** DNA alignment from ClustalX (Thompson et al., 1997) shows a balanced frame-shift mutation. **B.** RevTrans (Wernersson and Pedersen, 2003) back-translation of an amino acid alignment from ClustalX. The nucleotides have been shifted to fit the amino acid positions. Their positions do not correspond to the homologous nucleotides anymore.

# CONCLUSIONS

The use of DNATagger does not require installation and it is platform independent. It is available as an item of web-based free and open source software (FOSS). The entire process occurs in the browser of the user's computer and, once loaded, works off-line. It does not need to communicate with the server, and there is no file download or upload. The next step is to implement a function to export the colored alignments to a file for use in presentations and publications.

Genetics and Molecular Research 7 (3): 853-860 (2008)

# Availability and requirements

The DNATagger web interface can be found at http://www.inf.ufrgs.br/~dmbasso/ dnatagger/. We highly recommend the use of a "standards-compliant" web browser, such as Mozilla Firefox, Google Chrome, Konqueror or Opera. The application is platform independent, having been developed under Linux and tested under Mac OS and Windows. A command line version of the program can be requested at dnatagger@gmail.com.

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Genetics and Molecular Research 7 (3): 853-860 (2008)