DNAssist, a C++ Program for Editing and Analysis of Nucleic Acid and Protein Sequences on PC-Compatible Computers Running Windows 95, 98, NT4.0 or 2000

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ABSTRACT

The size of sequence databases, the rapid rate of generating sequence data and the need to routinely construct recombinant DNA molecules in molecular biological research necessitate the frequent handling and analysis of nucleotide and protein sequences on a computer. Although several console or Web-based utility programs are available, the application of these programs generally requires reformatting the data when proceeding from one such program to the next. The acquisition of elaborate, integrated program suites is financially prohibitive to smaller laboratories. Here, we report the development of DNAssist, a shareware program for editing and analysis of nucleic acid and protein sequences. It was developed as a multiple-document interface program—similar to a word processor—where sequences are entered, edited and analyzed in a single integrated environment. DNAssist can calculate the physicochemical properties of a sequence, convert between nucleic acid and protein sequences, translate DNA in multiple frames, identify open reading frames and locate ambiguous sequence patterns allowing gaps and mismatches. DNAssist also performs restriction enzyme and transcription factor-binding site analyses of DNA sequences, the multiple alignment of nucleic acid and protein sequences and the analysis of DNA sequences for nucleosome positioning sites.

INTRODUCTION

Research in molecular biology generally requires microcomputers to edit, manipulate and analyze nucleotide and protein sequences. The availability of the sequence of the entire genome of some organisms (3,20), the rapid generation of sequence data and the requirement to design and construct re-

combinant DNA molecules in research have made it necessary for almost all molecular biological laboratories to store and analyze sequences on computers. Common analyses include the identification of specific and functionally significant subsequences, the conversion of one sequence type to another, the location of consensus sites such as those for restriction enzymes or for transcription factors and the multiple alignment of sequences. Often the physicochemical properties such as molecular weight, melting temperature, isoelectric point, molar absorption coefficient at a given wavelength and the composition of polynucleotides and polypeptides are also required.

These analyses can be performed with individual utility programs that are often console-based (19), form-based Web interfaces to server-side Perl or C scripts (22) or with larger program suites such as GCG (7) that require mainframe or workstation computers running UNIX® or Solaris® operating systems. However, when performing a series of analyses with separate utility programs, the data are generally required or generated in specific formats and need to be reformatted in a text-based editor when proceeding from one analysis program to the next. The acquisition of sophisticated program suites that offer an integrated environment for analysis is often financially prohibitive to many smaller research laboratories. Here, we report the development of DNAssist, a shareware C++ program designed with a multiple-document interface (MDI) with integrated editing and extensive analysis capabilities that can be used on a PC-compatible computer running the Microsoft® Windows® 95, 98, NT® version 4.0 or 2000 operating system.

MATERIALS AND METHODS

Program Design

DNAssist is coded in C++ (1,18) and the graphic user interface developed using version 5.02 of the Borland® Objects Windows Library (Inprise, San Jose, CA, USA). The code was compiled with version 5.02 of the Borland® C++ compiler (Inprise) for Microsoft Windows 95, 98, NT4.0 or Windows

Sequence Editor

There are four different editor-type windows in which to display and edit DNA or degenerate DNA, RNA or protein sequences. New editor windows can be opened from the File menu or from the toolbar. The sequence editor window depends on the type chosen by the user from the menu, in the case of a new editor window, or on the sequence type of a file opened from a disk. For sequence files that do not specify a sequence type, such as text or FASTA (15) format files, DNAssist assigns a type that recognizes the largest percentage of characters in the sequence file.

The user can enter a sequence from the keyboard or load a sequence from a disk if the file is in text, FASTA (15), GenBank® (16), GCG (7), DNA Strider (13) or DNAssist format. The editor window responds to all uppercase or lowercase character keys that represent legitimate symbols for the window sequence type, general editing keys such as Delete and Backspace and the navigation arrow buttons. In addition, several shortcut keystrokes for common editing actions such as Copy, Cut and Paste are also defined. Alternatively, sequences can be dragged and dropped within (or between) compatible editor window types or pasted from the clipboard into a compatible window. Selections in a sequence can be made using the keyboard or the left button on the mouse and dragging the pointer within an editor window. The user can specify the font face and size of installed fixed pitch fonts, as well as the line length displayed in an editor window from the Options menu. All editor and analyses settings specified by the user are saved in a unique subkey of HKEY_CURRENT_USER in the Registry when the user exits the program, a feature that allows DNAssist to be used in a multi-user environment.

A sequence displayed in an editor window is manipulated in memory as a continuous series of eight-bit character data types and is managed by a specialized C++ class. This class will dynamically expand or shrink the allocated memory block in which the character array is placed in response to additions or deletions to the sequence. This allows optimum memory use and the manipulation of sequences with a size constrained only by the physical configuration of the computer.

Figure 2. Sequence conversions and translations in DNAssist. (A) Nucleic acid sequences can be reversibly interconverted or converted to a protein sequence from the Convert menu. (B) DNA sequences can be translated in 1, 3 or all 6 reading frames from the Analyze menu.

Sequences can be saved to disk in ASCII text or DNAssist format. The format file of the latter contains a file header of five semicolon-delimited fields, in which information regarding the stored sequence is given and is followed by a series of bytes that correspond to the ASCII character values of the nucleotides or amino acid residues in the sequence.

Sequence Analysis

The analysis methods that can be selected from the main menu or the context-sensitive pop-up menu depend on the sequence type of editor window with input focus. The menu entries and toolbar icons of analysis methods that cannot be applied to a given sequence type are dimmed to present the user with a more intuitive graphic interface.

The result of an analysis is displayed in a result MDI window. In the case of a text-based result, the content of the result window can be saved to a disk or copied to the clipboard in rich-text format (RTF), from which it can be opened or pasted into other applications that recognize this format. Similarly, graphic results can be saved or copied and imported into other applications that recognize the enhanced Windows metafile format. The user has extensive control over the format in which text-based results are displayed. In the case of sequence listings, one or both DNA strands can be shown as a continuous

Figure 3. Restriction enzyme analysis. All restriction enzymes in a specified file are listed in the Restriction Enzymes dialog box. Individual restriction enzymes can be deleted, new enzymes added and the modified file saved to disk. The user can also select specific restriction enzymes from the enzyme list.

Figure 4. The display of a restriction enzyme analysis. A graphic map of a restriction enzyme analysis will display all unique sites and, if specified by the user, will indicate the presence of a cleavage site for a selected restriction enzyme, with an asterisk.

sequence or grouped into sequence blocks with a block size and block spacing that may be specified. Sequence numbering can be displayed above, below or to the right of such listings.

Sequence Properties

DNAssist can calculate and display the general physico-chemical properties of nucleic acid and protein sequences, plus the hydrophobicity (12), hydrophilicity (11) and antigenicity (21) profiles of protein sequences. The following are displayed for nucleic acid sequences: the number of each of the four nucleotides, G+C%, A+T%, sequence length, melting temperature calculated by the nearest neighbor method (6), annealing temperature, molecular weight, molar absorption coefficient and $E_{0.1\%}$ at 260 nm (2). Displayed for protein sequences are the number and mole percentage of each of the 20 amino acid residues, sequence length, molecular weight, pI (4), molar absorption coefficient and $E_{0.1\%}$ at 280 nm (14). These properties can be displayed for the entire sequence or for only a selected subset of the sequence.

Sequence Reversals, Conversions and Translations

A DNA sequence can be transformed between a linear and circular configuration or converted to its complementary

sequence in the 5' to 3' orientation. This conversion takes place as a combined reverse-complement operation. A nucleic acid sequence can also be converted to a protein sequence, DNA and RNA sequences can be interconverted (Figure 2A) and a DNA sequence can be translated to a protein sequence in one, three or all six frames (Figure 2B). In addition, a protein sequence can also be reverse translated to a degenerate DNA sequence. Nucleic acid to protein conversions and translations are performed according to the entries in the codon table selected from the Options menu.

Codon tables generated by the GCG program, Codonfrequencies (7), are compatible with DNAssist. A translated sequence is displayed as a nucleotide sequence listing aligned with the single-letter amino acid abbreviation. Stop codons are indicated by asterisks. If an in-frame stop codon is encountered during a nucleic acid-to-protein conversion, the user is alerted to the truncation of the protein at the given position.

Finding ORFs and Patterns

All the sequence searches in DNAssist make use of a class implementation of the Boyer-Moore algorithm (5). Apart from routine substring searches from the Edit menu, DNAssist also allows searching of a sequence for ORFs and se-

quence patterns. The ORF search function uses the start and stop codons in the selected codon table to search for continuous sequence stretches that may code for proteins. The pattern-search function searches for a defined sequence pattern, allowing ambiguous nucleotides or amino acid residues in the pattern and a user-defined number of sequence gaps and mismatches. The sequence of an identified ORF or pattern match is highlighted in the editor window.

Restriction Enzyme Analysis

DNAssist performs a restriction enzyme analysis using restriction enzyme data from a Rebase database file (17). Updated versions of this enzyme data file can be downloaded from the Rebase Web site at <http://rebase.neb.com/rebase/rebase.files.html>. All or a selection of the restriction enzymes in the file can be used (Figure 3), and enzymes leaving a specific type of cleaved terminus can also be specified from this set. The result of a restriction enzyme analysis can be displayed as a graphic map (Figure 4), a sequence listing and in tabulated format. The tabulated restriction enzyme analysis lists the enzymes used in the analysis, the number of times that each enzyme cleaves, the cleavage positions and fragment sizes in descending order and a list of restriction enzymes that do not have recognition sequences in the analyzed sequence.

Predicting Nucleosome Rotational Positions

The propensity of a DNA sequence to accommodate a rotationally positioned nucleosome is calculated with the Drew-Calladine algorithm (8) and is displayed as a plot of the probability of finding a rotationally positioned nucleosome at every position of the analyzed sequence.

Sequence Alignments

DNAssist conducts pairwise and multiple alignments of DNA, degenerate DNA, RNA and protein sequences by constructing a phylogenetic tree that is based on a matrix of all pairwise sequence similarity scores, as used in CLUSTALW (10,19). The alignment allows ambiguous nucleotides or

Figure 5. Multiple alignments in DNAssist. A multiple alignment is displayed with conserved and identical amino acid residues or identical nucleotides, as shown in a selected font foreground and font background color. The minimum number of nucleotides or amino acid residues that must be identical or conserved to be indicated as such can also be specified.

amino acid residues. Aligned sequences are displayed as sequence lists with identical nucleotides and amino acid residues or as conserved amino acid residues, indicated in specified font foreground and background colors (Figure 5). From the Options menu, the user can specify the colors and the minimum level of sequence identity or conservation required before residues are indicated as such. The user can also specify Gap extension, opening penalties and the minimum level of divergence before alignment is delayed. DNA sequence alignments can be performed with a user-selected DNA weight matrix, and the penalty associated with a non-identical purine-purine or pyrimidine-pyrimidine alignment can be set. For protein sequences, the protein weight matrix and penalties for opening gaps at specific residues, sequence termini or hydrophilic regions are also user selectable.

Promoter Analysis for Transcription Factor Binding Sites

A DNA sequence can be analyzed for the presence of transcription factor binding sites listed in the Transfac database (9). DNAssist lists transcription factors in the database in a hierarchical manner, grouped by kingdom, species and factor. These groups are displayed in list boxes with a master-slave relationship. For example, the user can select all vertebrate factors or only the factors identified in *Homo sapiens*. DNAssist issues a local structured query language (SQL) query against the Transfac database and displays only the factors returned for the selected kingdom(s) and species. The user can then choose to perform the analysis with all or only a selection of displayed transcription factors. Updated versions of the Transfac database can be downloaded from <http://transfac.gbf.de/TRANSFAC/index.html>. The result of a transcription factor analysis can be displayed as a graphic map, sequence listing or in tabulated form, where the name and binding position of the selected transcription factors are given.

SUMMARY

DNAssist is an excellent tool set for the molecular biologist while remaining affordable for the research scientist. As such, it is a marked advance over other such tools on the market that cost as much as 10 times more. In today's atmosphere of increasingly tight funding, DNAssist is an essential and cost-effective program for almost all molecular biology researchers.

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