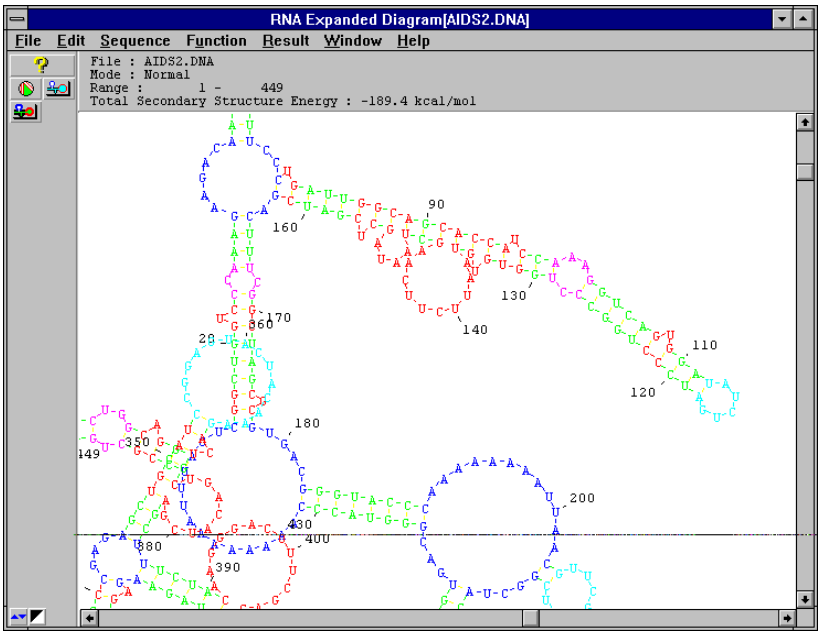
A View Through
DNASIS® 2.5 for Windows™



Introduction

DNASIS for Windows is an easy to use, comprehensive package for DNA and Protein Sequence Analysis on your IBM PC compatible computer. It provides the clear and understandable graphic user interface that you would expect from the Windows software. This *Journey* demonstrates the features of DNASIS 2.5 for Windows and how it can help in your research. We believe that after you try DNASIS for Windows, you'll see that it can be a valuable asset for your everyday lab work.

DNASIS 2.1 for Windows Features

- BLAST search
- Windows 95 and NT compatible
- Circular restriction maps
- Restriction enzyme searching with maps
- Dot matrix similarity comparison
- Multiple sequence alignment
- Sequencing project management
- Open reading frame search
- Digitizer entry with voice read back
- Coding region prediction
- Enhanced primer design
- tRNA prediction
- Phylogenetic trees
- Homology plots
- Helical wheels
- Hydrophobicity and hydrophilicity
- RNA folding structure prediction
- Mutational site search
- Database similarity searching
- Database keyword searching
- DNA and Protein Motif Analysis
- Protein structure analysis
- Proteolytic enzyme
- Protein isoelectric point prediction

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Getting Started

Loading DNASIS

DNASIS for Windows should be contained in a folder called WDNASIS which contains both the program functions and sample data. The DNASIS setup program runs in the Windows environment. The program is installed in the following steps.

Step 1. Quit active applications before installing DNASIS for Windows Demo.

Step 2. From the windows program manager, select **Run…** from the **File** menu of the Program Manager in Windows 3.1x or under the Start button in Windows 95 or NT v4.0.

Step 3. Type the following in the command line: **(Your CD-ROM drive letter):\dnasis25\dnademo\setup.exe**

Step 4. Choose Windows 3.1 or Windows 95/NT. The DNASIS demo will be installed in the C:\wdnasis directory. If you would like to install the program in a different directory you may indicate it now.

Step 5. Follow the prompts on the screen to continue installation. If you are unable to complete the BLAST program setup portion of the installation (this may occur if the NCBI server is busy), please run the BLAST setup program later by clicking on the BLAST Setup program icon.

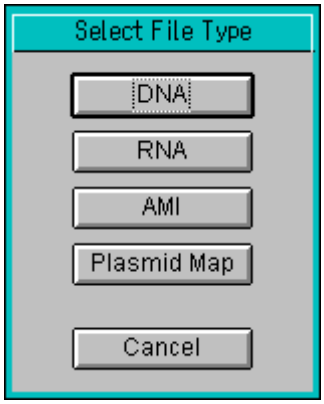
NOTE: Your computer must be configured to communicate using the TCP/IP protocol in order for the BLAST Search to work properly. Please check with your system administrator if you have questions about your system configuration. The BLAST Search function is not available in Windows 3.1x..

Entering and Editing a Sequence

All new data is entered into DNASIS via standard Windows procedures. However, there are some differences since more than one type of "new" file may be entered.

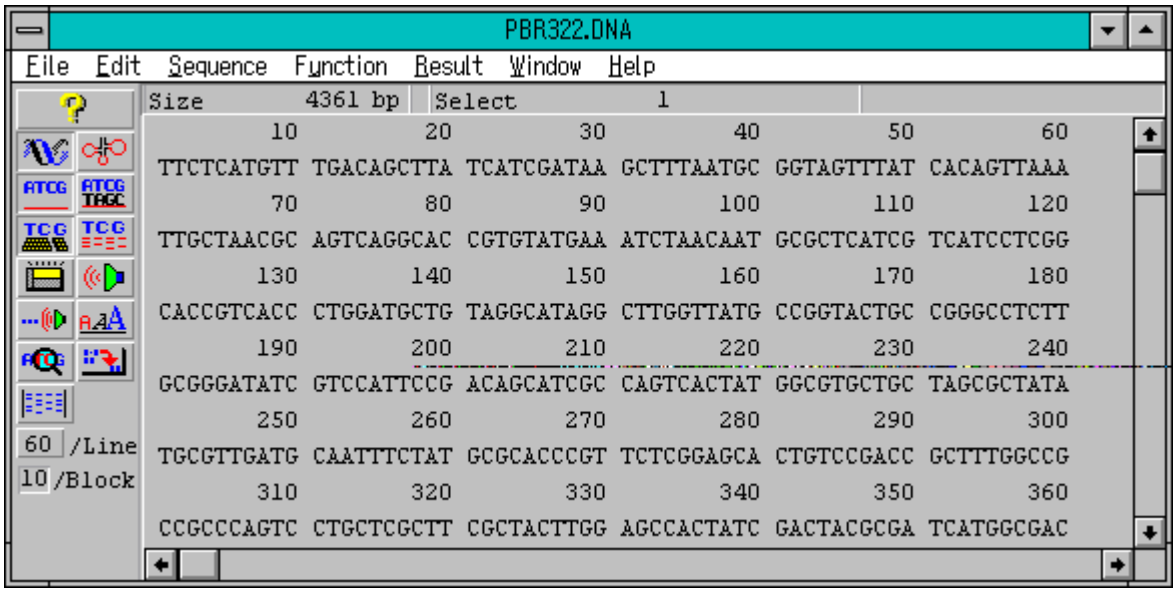
1. **From the File menu, choose New.**

   Click on the type of file you wish to create. A new file with the name "Untitled" will open



   This is the editing window. From here, you can enter your sequence.

2. **Note that many functions on the top menu bar have been duplicated as icons on the side bar.**



DNA

 The DNA icon is an indicator switch. When it's turned on, the file sequence contains thymine (T) instead of uracil (U).

RNA

 The RNA icon is an indicator switch. When it's turned on, the file sequence contains uracil (U) instead of thymine (T).

Protein

 When an amino acid (AMI) file is open, the protein icon is displayed and the DNA and RNA icons are suppressed.

Single strand

 The single strand icon displays the file sequence as a single stranded nucleic acid.

Double strand

 The double strand icon displays the file sequence as a double stranded nucleic acid.

Entry

 The entry icon activates sequence input from the keyboard.

Proofreading

 The input mode (compare) icon activates the proofreading function. This function is useful for verifying the accuracy of sequence that has been entered from the keyboard or from a digitizer.

Information

 The information window icon opens an editable text box in the top portion of the sequence editor screen. Here you can annotate the sequence file with memo information, references and notes.

Audio confirmation

 The speech output icon activates the audio confirmation feature of DNASIS. This feature may be used in conjunction with proofreading. A sound card must be installed for this function.

Audio confirmation options

 The speech options icon brings up a dialog presenting choices for the voice read back feature, discussed in detail in "Sequence Entry", below.

Digitizer

 The digitizer icon activates sequence input from the Hitachi HDG-1717BL or compatible model digitizer. This icon is duplicated by the "Digitizer On..." command in the Sequence menu.

Search

 The search icon brings up a dialog box in which you specify up to five query (key) sequences to search the file.

Jump

 The Jump to icon allows you quickly to move the pointer to any position in the file. Clicking this icon brings up a dialog box in which you specify the sequence position to move to. This icon is duplicated by the "Jump to" command in the Sequence menu.

Font size change

 The Font size change icon allows you to change the size of letter which is displaying the nucleic acid or protein sequence.

You can call up definitions for icons throughout DNASIS using on-line help under the

 **menu or by clicking and holding the right button of your mouse over the icon.**

Restriction Enzyme Search and Plasmid Map Drawing

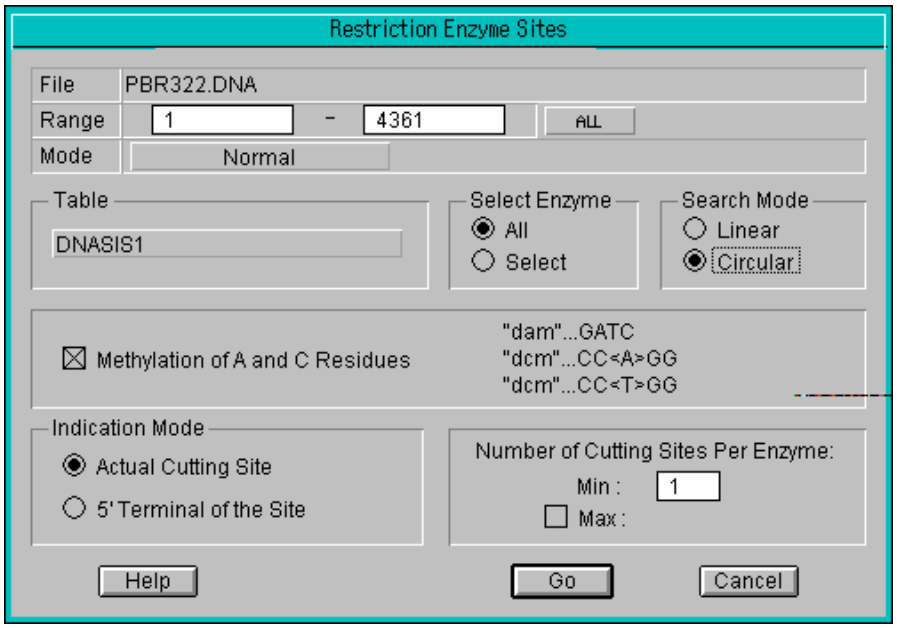
This section will demonstrate how DNASIS searches for restriction enzyme sites in DNA sequences.

Restriction Enzyme Search

1. **From the File menu, choose Open.**
2. **Find and double-click on the folder named Sample Data.**
3. **Select the sequence named pbr322.dna and click OK.**
4. **From the Function menu; go to the Search function and select Restriction Enzyme.**

   You will get a dialog box for specifying site search parameters or selecting customized enzyme tables. **Choose the parameters shown in our example below.**

5. **In the bottom of the dialog box at *Number of Cutting Sites Per* set *Min:* at 1, and *Max:* at 2.** These parameters will show enzymes that cut a minimum of 1 time and no more than 2 times.



6. Click on the Select button of Select Enzyme.

You can select All, or you can specify enzymes from a listing of enzymes in our file.

7. **First, select None; then check the 6-Cutters box and the Blunt End box; now click on Add and then OK.**

   You have selected all blunt end cutters of 6-base recognition sites.

8. **To run analysis, click the Go button.**

   You will then get the Restriction Site Table. The table shows the enzyme, the recognition pattern and the start position of each cutting site.

9. **Display the linear sequence map by selecting the**  **icon.**

   Cut sites are indicated by carets. The relevant enzymes are listed under the carets. Possible methylation sites are indicated by asterisks above the sequence.

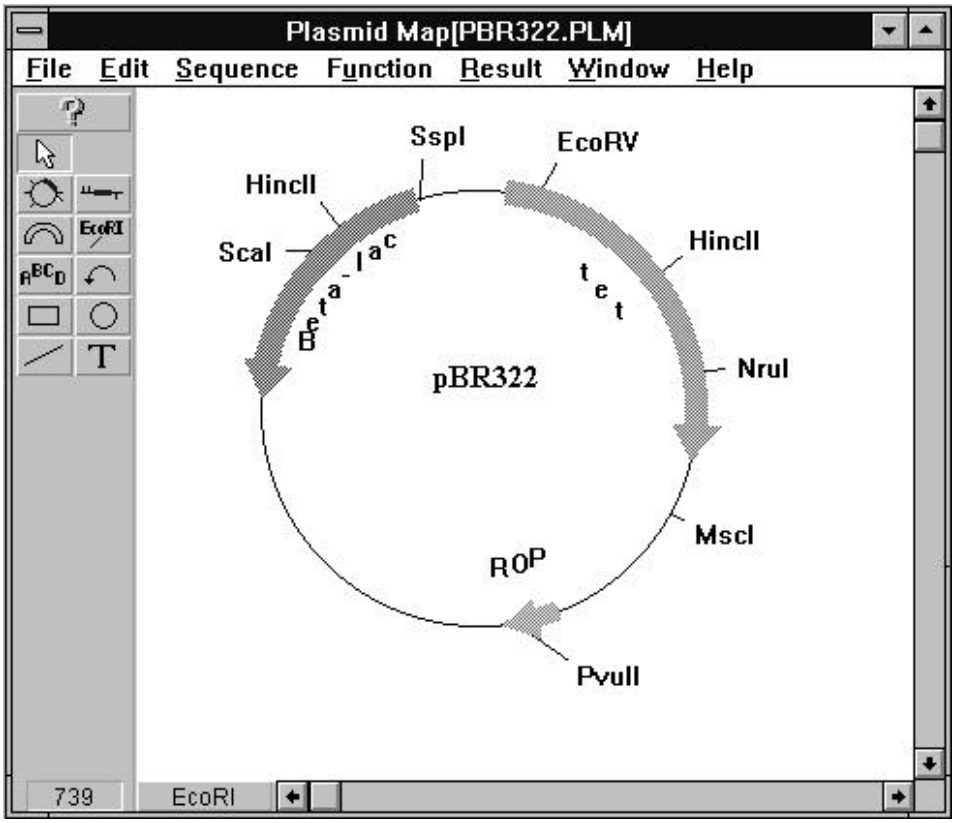
Plasmid Map Drawing

DNASIS gives you the ability to create illustrations for your lab notebook or publications.

1. **From the sidebar menu, select the plasmid map display**  **icon.**

   This will create a plasmid map in a new drawing window. You can move the map with the mouse by clicking and dragging. You can also move the individual enzymes by first de-selecting the map (click the mouse outside the circle) and then selecting an enzyme.

   The lower left hand corner indicates the base number currently active.



   To change colors, patterns, sizes or fonts, select any object, then go to the RESULT function and make changes.

   To Add regions of interest, select the drawing icon  . Hold it down and choose the Plane or Arrow. Go to the plasmid and hold the cursor down while dragging it along the circle.

2. **You can experiment with the other plasmid drawing tools by clicking on the icons in the side-menu bar.**

3. **When finished, this image may be saved and printed for your lab book.**

Protein Coding Region Prediction

This function scans the selected sequence for codons known to be associated with initiation and termination of transcription. You may designate any combination of start codons. Stop codons are prescribed (i.e.., amber, ochre, umber).

Coding region prediction is with Fickett's method. This method finds open reading frames with strong probability to code for protein. Fickett's method is based on two findings: base distribution in coding sequence codons is not random and coding sequence tend to have higher GC content.

Depending on which organism you are working with, DNASIS also provides you with a large selection of codon preferences. Gene composition is known to differ from non-coding sequences. DNASIS can measure a known representative gene against its target unknown sequence and compare their sequence composition.

Relative frequencies of the types of codons and amino acids translated from them are also calculated in the Protein Coding Region Prediction function. A codon frequency table is created that displays the relative amounts of the nucleotides which make up positions in a codon.

1. **From the File menu,choose Open.**

2. **Find and double-click on the folder named Sample Data.**

3. **Select the sequence named aids1.dna and click OK.**

4. **From the Function menu, go to Prediction and select Protein Coding Region...**

   This opens a dialog box for setting prediction parameters. From the parameter dialog box you can set options such as codon preference table, Init/Term codons, and Fickett's method for coding region prediction.

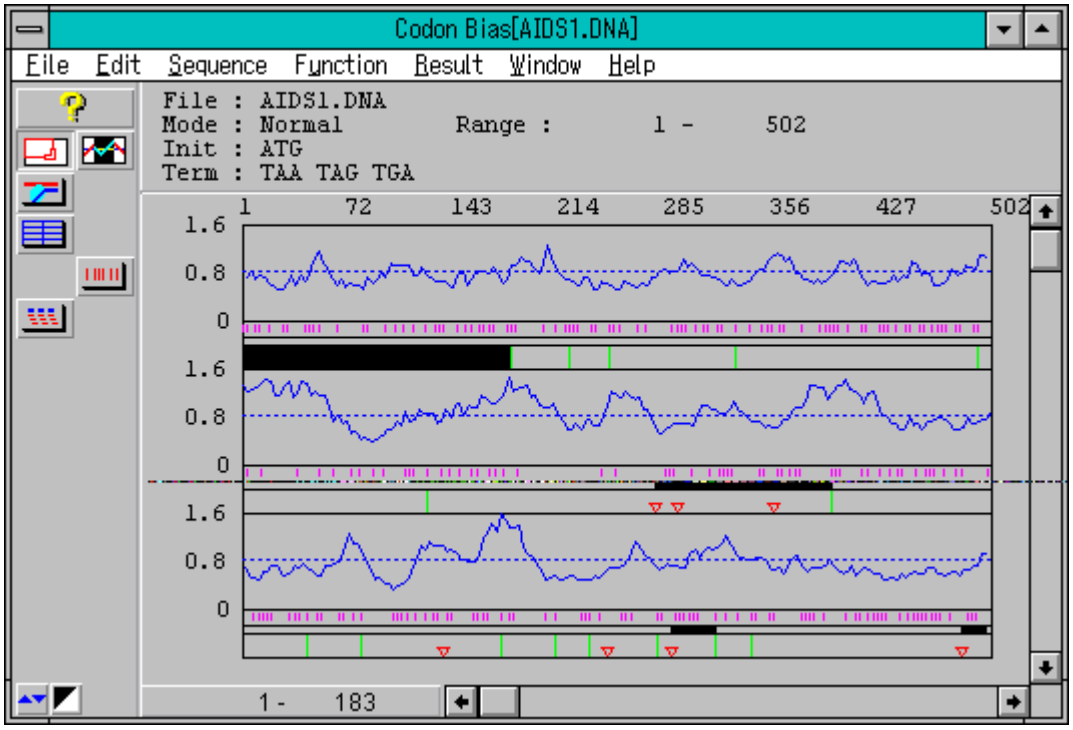
5. **Click the Go button.**

   The graph of AIDS1.DNA sequence will appear showing initiation and termination sites of the three reading frames.

   Initiation codons are shown as triangles and termination codons are shown as vertical lines across the sequence.

   Codon bias output is indicated as a trace showing the relative likelihood of a codon occurring in a gene as opposed to a random sequence.

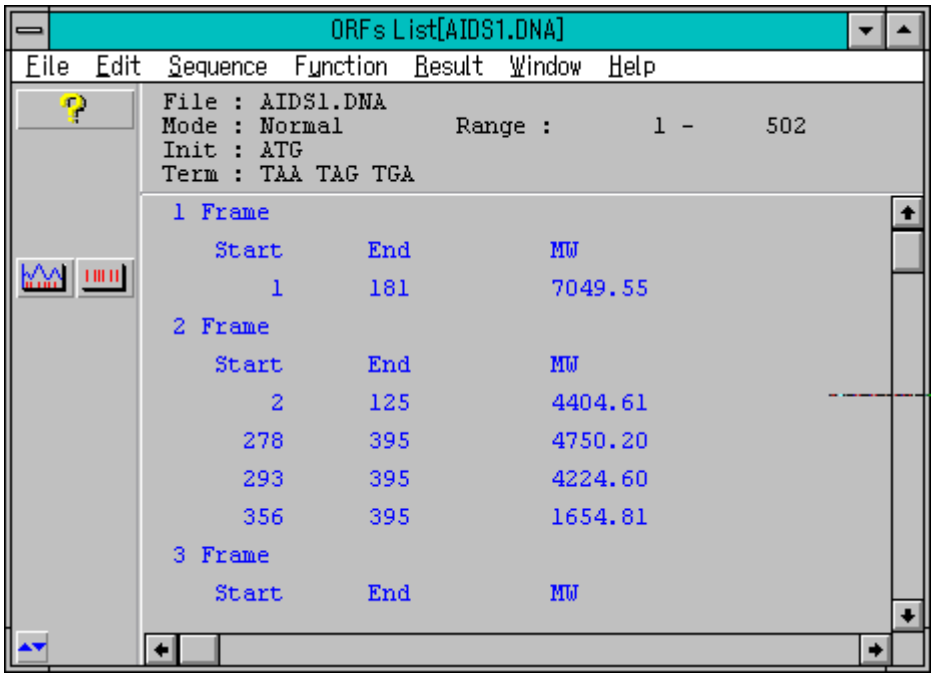
   You can expand or zoom in on the diagram. By clicking and dragging the arrow  at the corner of the display, you can enlarge the display. By selecting the zoom icon  , you can drag the mouse across a region in the display and zoom expand on the region. Double click on the zoomed image to return to the original size.



6. **Be sure that the arrow icon is selected and move the mouse and select an initiation site.**

   Note that as you select sites, the termination site will be adjusted.

7. **Select the Position Table icon**  **on the side bar menu.**



This table lists the initiation and termination for each frame in a tablet format along with molecular weights.

Mutational Site Analysis

Single base mutations are the easiest way to create and introduce a restriction enzyme into your sequence. The mutational site analysis function searches for a single base mutation which will create a new restriction enzyme site. This application will analyze and display for silent mutations on each of the three reading frames.

1. **From the File menu, choose Open.**

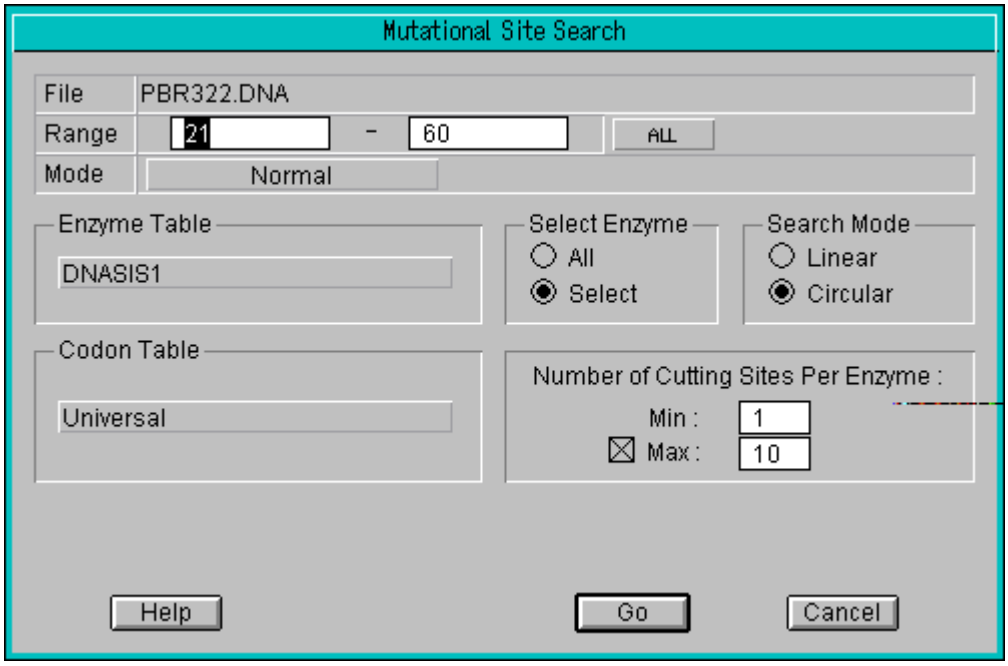
2. **Select the sequence named pbr322.dna and click OK.**

3. **Select bases 21-60 with the mouse.**

   This will be the range of the mutational site analysis.

4. **From the Function menu, go to Search and select Mutational Site Search...**

   A dialog box that is very similar to the Restriction Enzyme search will appear. Within this dialog box, you may select enzymes that you would like to introduce, enzyme table, number of cut sites and codon table.

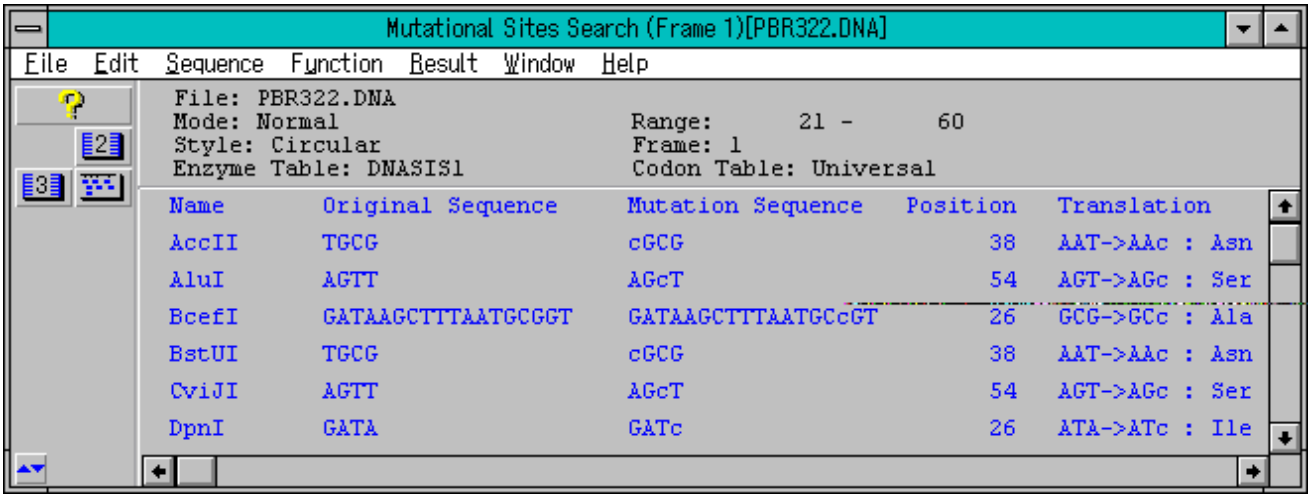


5. **Click on the Select button of Select Enzyme.**

6. **Select all enzymes and click OK.**

7. **Begin analysis by clicking the Go button.**

   You will then get a Mutation Sites Table of results. This table displays the results of all the single base mutations that can be introduced to create a new restriction site without altering the corresponding reading frame.



To display the results from the other two reading frames, click the appropriate reading frame icon  or  .

To display all of the potential restrictions sites, click the sequence display  icon.

You have now completed the demonstration for mutational site analysis. You can close these windows and continue with the demonstration or just exit the program.

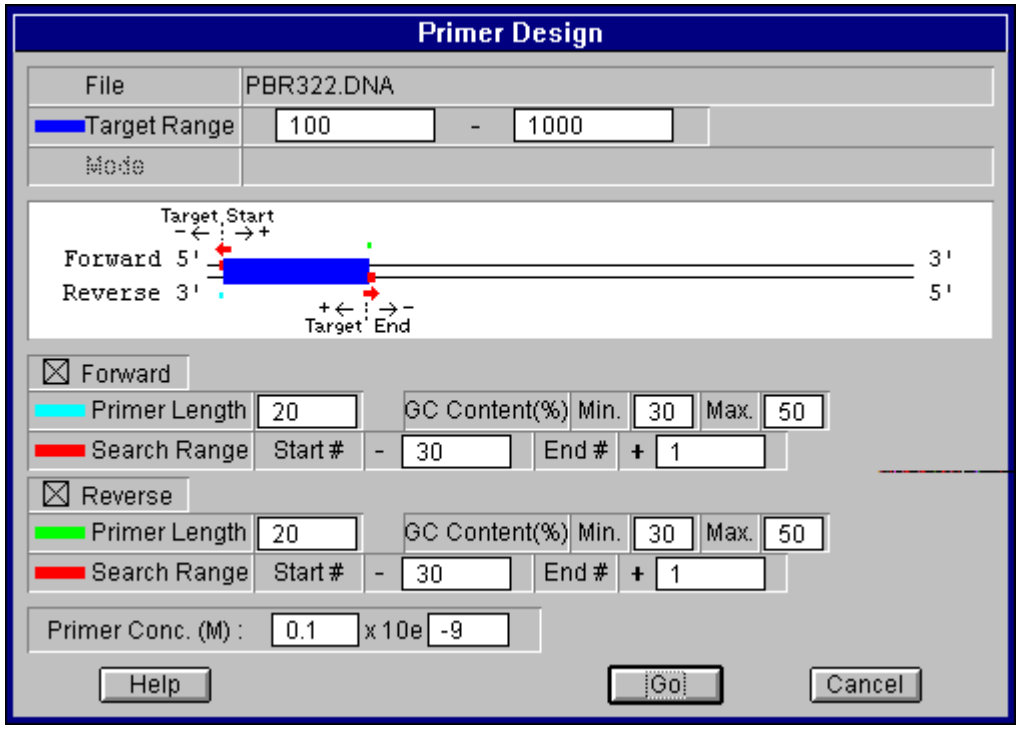
Primer Design

1. **From the File menu, choose Open.**

2. **Select the sequence named pbr322.dna and click OK.**

3. **From the Function menu, go to Search and select Primer Design**

   A dialog box appears for setting primer design parameters. The display shows the selected regions for amplification and for priming. Note: Arrows indicate the direction of primer search, not the direction of a primer amplification.



4. **Set the Target Range at 100-1000**

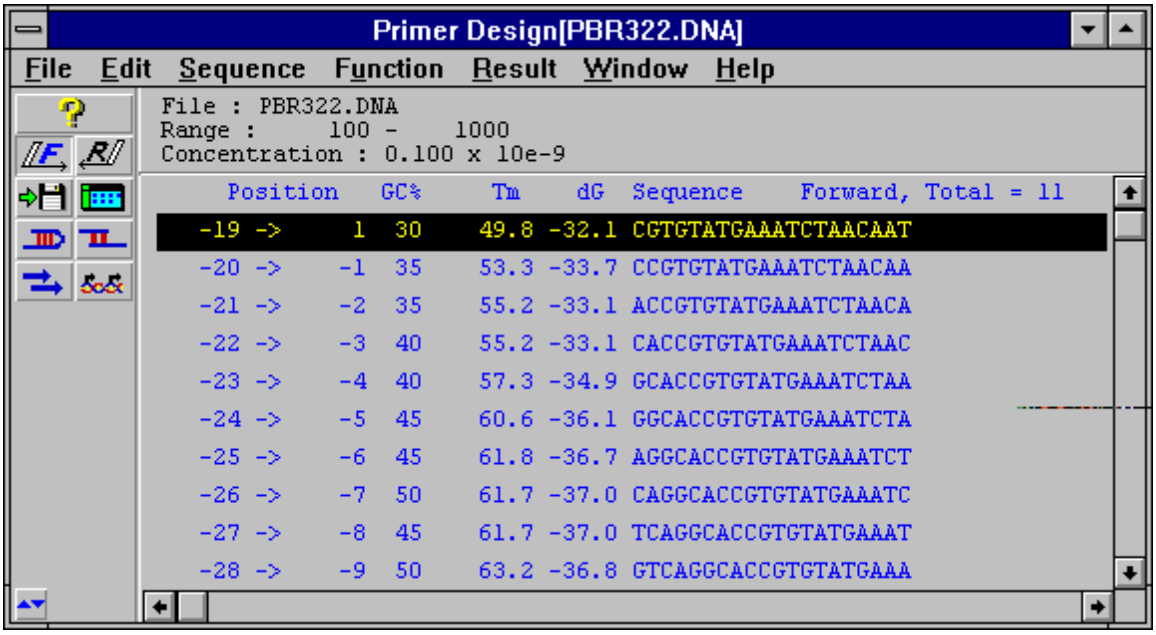
5. **Be sure that both the forward and reverse primer boxes are selected.**

   Set the parameters to match the above example.

6. **Set the Search Range**

   This is the distance searched on both sides of the highlighted region for the primer. In this example, base 100 is the neutral 5' start position. Bases to the left are referred to as negative numbers. The further upstream, the negative values increase. The 3' end is similar. In our example, base 1000 is the 3' neutral position, base 1030 is considered -30.

7. **Click Go.**



This window shows a listing of potential forward primers including the distance from the origin of the search, the length, the GC content and the Tm (melting temperature).

-19, positions the 5' end of the primer at base 81. 19 bases to the left of base 100

8. **From the sidebar menu, select the icon .**

   This will give you a screen with the list of potential reverse primers.

9. **Select the icon to display a list of hairpin turns found for the selected primer.**

10. **Select the icon to display a list of possible dimers for the selected primer.**

11. **Select the icon to display a list of repeats for the selected primer.**

12. **Select the icon to display a list of false sites for the selected primer.**

13. **Select a primer for further analysis.**

    Highlight the primer of choice, then select **Result** from the top menu bar. Select **Open Editor** or double click on the primer of choice. This will enable you to open the individual primer for primary or secondary analysis.

You have now completed the demonstration of the primer design functions. You can close these windows and continue with the demonstration or exit the program.

RNA Structural Prediction

1. **From the File menu, choose Open.**

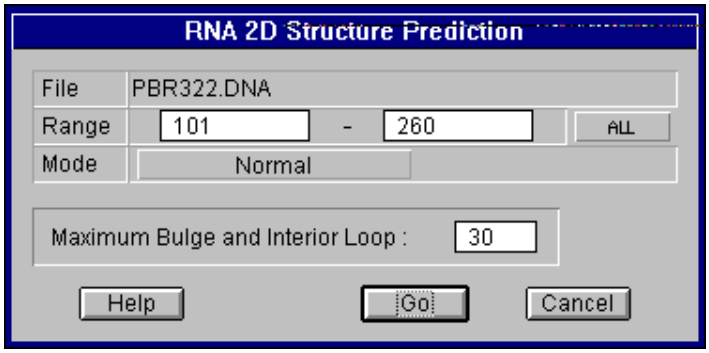
2. **Select the sequence named aids1.dna and click OK. Convert to RNA by clicking on the RNA icon**  **.**

3. **Select base from 101 to 260 with the mouse.**

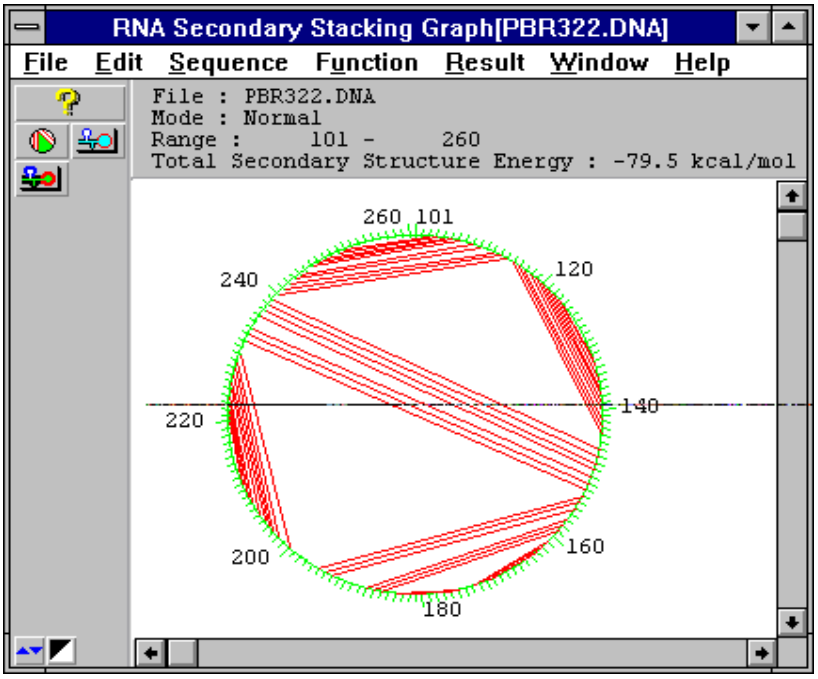
   This will be the range of the RNA folding analysis.

4. **From the Function menu, go to Prediction and select RNA Secondary Structure.**

   This will open a dialog box which allows you to change parameters of the analysis.

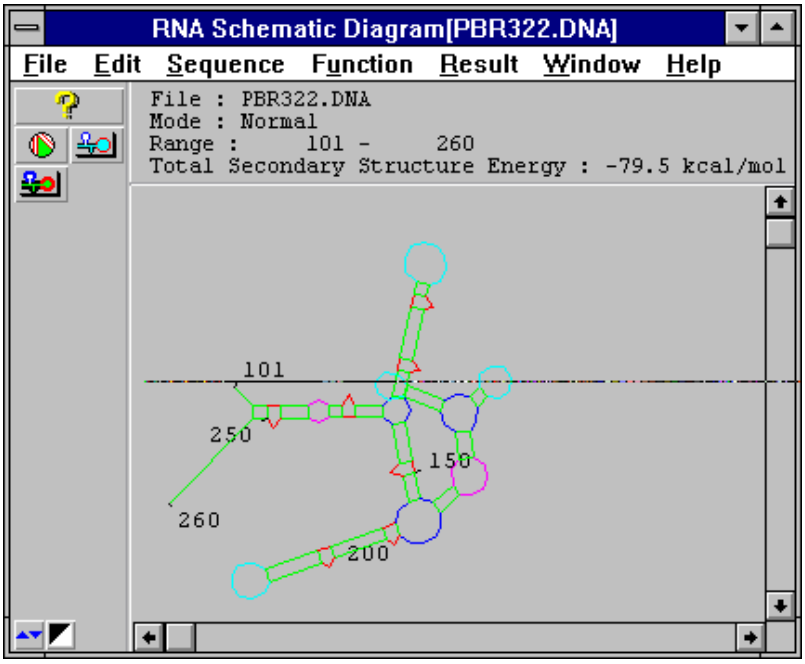


5. **Click the Go button to start the analysis.**

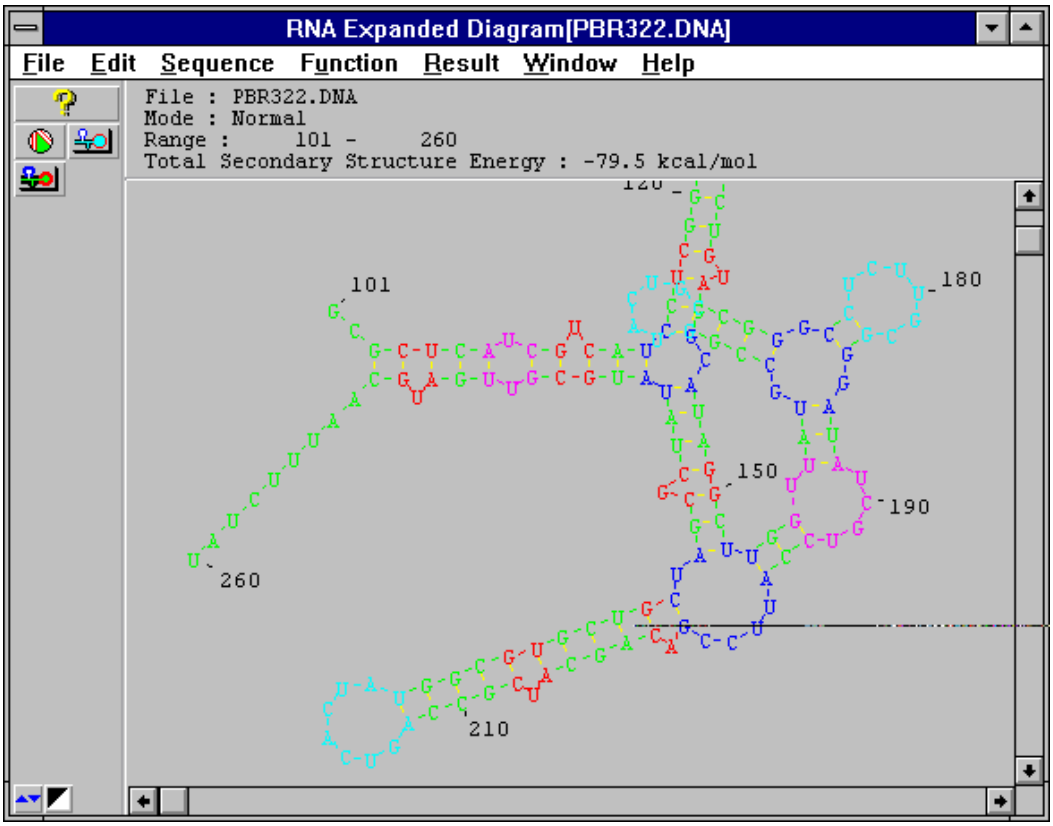


The window displays a stacking graph for the predicted RNA secondary structure. It is a circle graph of predicted base pairing.

6. **For a schematic representation click on the diagram button  from the side menu bar.**



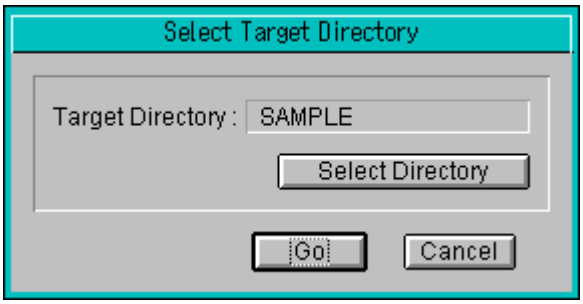
7. **To view an expanded diagram, drag the mouse to box the a rea of interest, click on the expand button  .**



Managing a DNA Sequencing Project

1. **From the top menu bar, go to Function and select Contig Manager.**

   Contig Manager is the portion of DNASIS that manages DNA sequencing projects. This program will assemble sequencing fragments into a consensus.



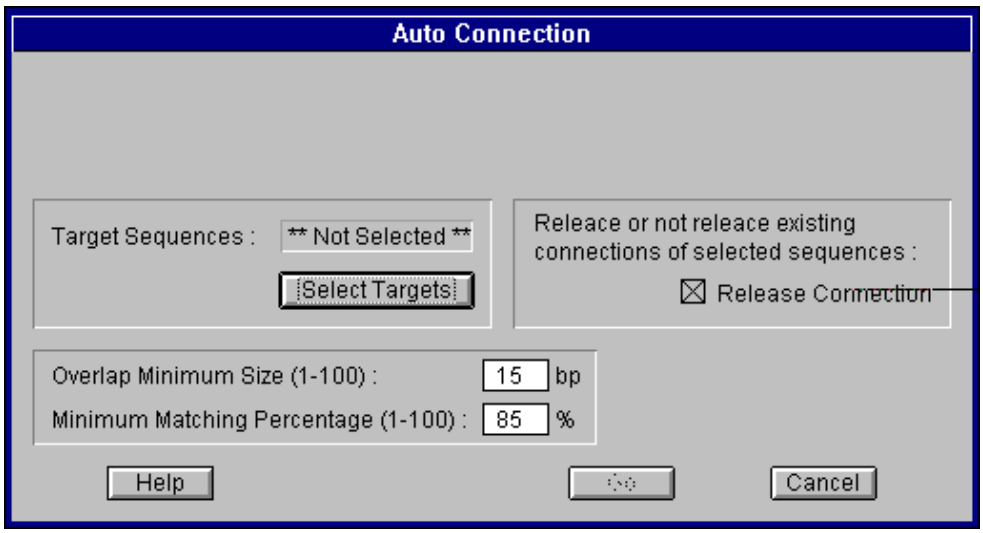
   Select the folder where your sequencing data resides. Our data is in the Sample Data Folder.

2. **Click the Select Directory button and open the Contig folder within the Sample Data Folder , select a sequence and click OK.**

3. **Click Go.**

   This will open all DNA sequence fragment files for contig assembly.

4. **From the side menu bar select the Auto button .**



   This is the parameter window. Select your target sequences from the Sample Data folder.

5. **Click on the Select Targets button. A Select Targets dialog box will open.**

6. **Click and select all the FRAG#.DNA files and then click OK.**

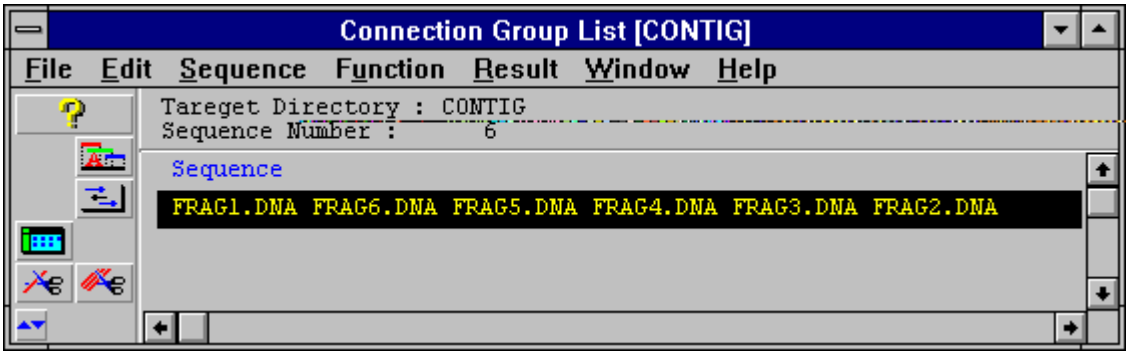
7. **Change the parameters for our example.**

   – The Overlap Minimum size should be set to 15 bp.

   – The Minimum Matching Percentage should be set to 85%

8. Click the Go button to begin the contig assembly.

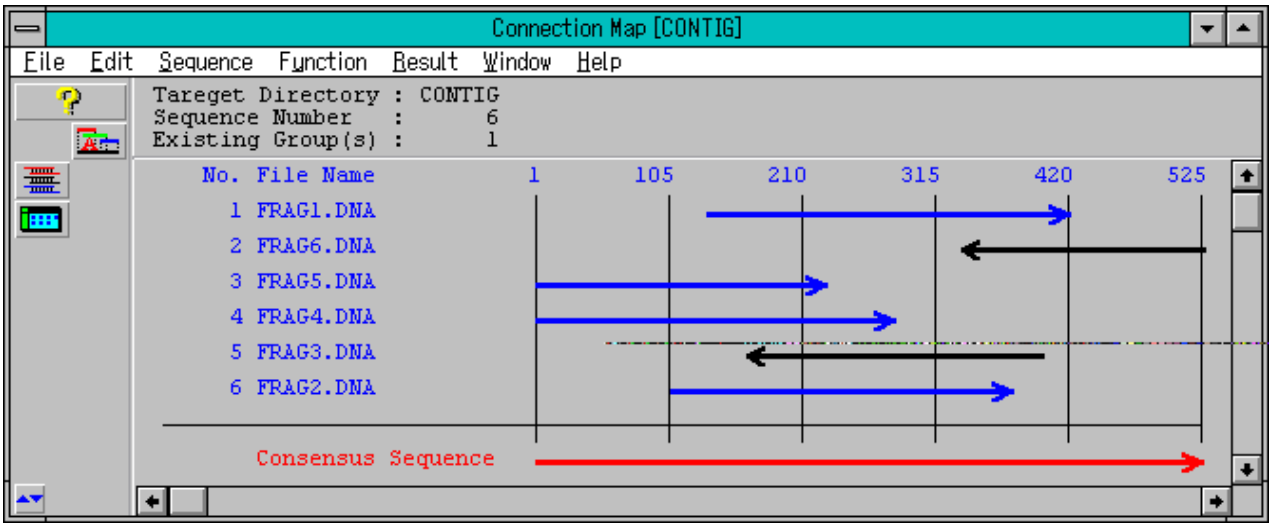
All the FRAG#.DNA files in this example should have connected into a single contig. This contig is now seen in the sequence list as one file with all of the fragment file names. The connected fragments should now be selected.



You may view or edit the connected sequences by clicking various buttons in the side menu bar.

9. Click on Map button  to get a strategy map of the Contig.

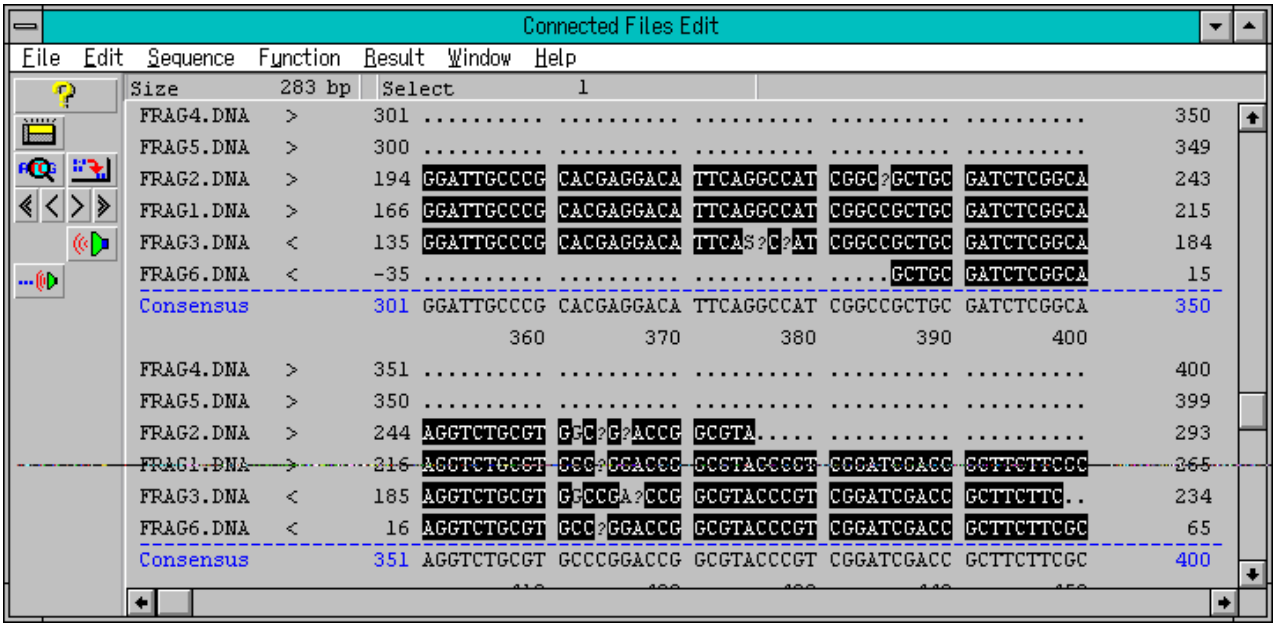
Each arrow represents a sequence fragment and the direction of the arrow describes whether it has been inverted and complemented or not.



10. To edit the contig, click the edit button on the side menu.

For this example, Choose Matching Bases to highlight regions of homology in the contig alignment editor. Choosing Unmatching Bases will highlight unmatching bases in the editor. Click **OK** to open up the editing window.

The shaded regions indicate regions of homology, gaps are indicated by question marks.



11. The sequences which you have edited and connected may be saved under the Connection Group List window.

To save an individual edited sequence, make sure the cursor is in the sequence of interest and choose the File menu. Choose either Save or Save a Copy As. To save the entire contig alignment including the consensus, choose Save List. To save only the consensus sequence, choose Save Consensus from the Result menu.

Note: The demo version of DNASIS does not support the save functions

Creating a Dot Matrix Plot (Harr Plot)

Dot matrix plots show similarities between two sequences. The plots allow you to visualize similar regions and find repeating regions in the sequences.

1. **From the File menu, choose Open.**

2. **Find and double-click on the folder called Sample Data.**

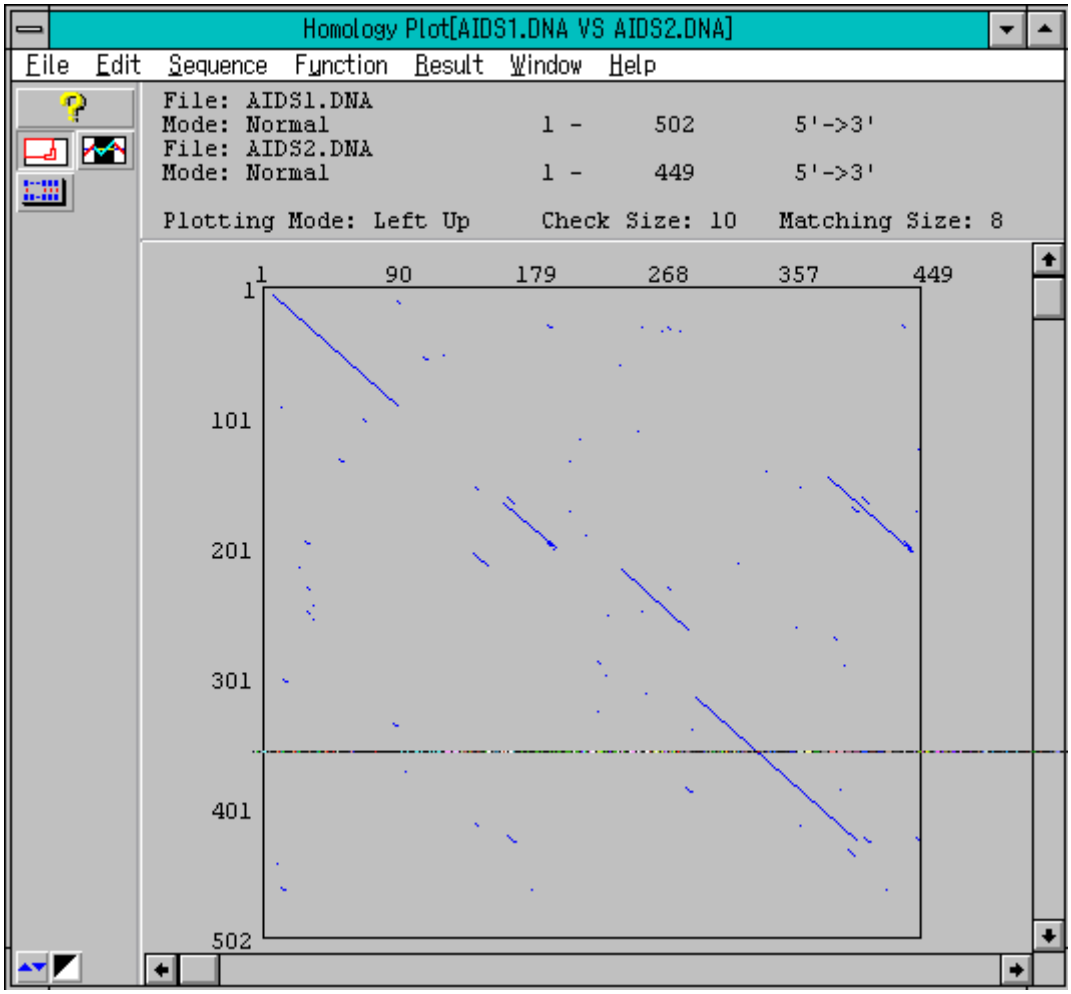
3. **Select the sequence named aids1.dna and click OK.**

4. **From the Function menu, go to Compare and select Homology Plot.**

   A dialog box will appear. From here, you select the target sequence to be compared against. This window also allows you to set various parameters.

5. **Click on the File Select button and choose aids2.dna and click OK.**

6. **Click on the Go button.**



After the program finishes calculating, a dot matrix homology window appears. Each diagonal represents a region of similarity.

Altering the Display

The side menu bar of the Dot Matrix window allows the user to change the display. You may alter the size of the entire plot or zoom in on a selected region of the plot.

- With the  button selected, you can enlarge the plot by dragging the lower right corner of the plot display box with the mouse.

- By selecting the  button, you can zoom in on specific regions of the plot. Use the mouse to select a portion of a diagonal. This will zoom in the selected area. Double clicking on the plot will zoom out.

- You can also display the pair-wise alignment of a diagonal. First, select the alignment butto  . Use the mouse to select a portion of the diagonal, the selected region will be aligned and displayed.

You have now completed the demonstration for creating a Dot Matrix Plot. You can close these windows and continue with the demonstration or just quit the program.

Multiple Sequence Alignment (Higgins)

DNASIS for Windows utilizes the Higgins and Sharp algorithm. The Higgins algorithm is based on a progressive alignment method where each sequence is compared to every other sequence. The sequences are progressively aligned according to a sequence based phylogenetic tree calculated from the initial comparison. The results from the Higgins algorithm is extremely fast and the alignments appear to be as sensitive as those from other available methods.

1. **From the Function menu, select Multiple Sequence. Click on the protein box and click OK.**

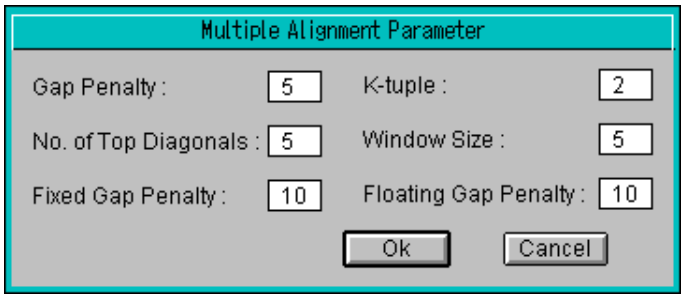
2. **A dialog box will appear, choose Add.**

3. **Find the globin sequence folder in the sample folder and click OK.**

   Add the first two (2) sequences and then add the last two (2) sequences by holding down the control key while selecting each sequence and click OK. This will bring you back to the first dialog box.

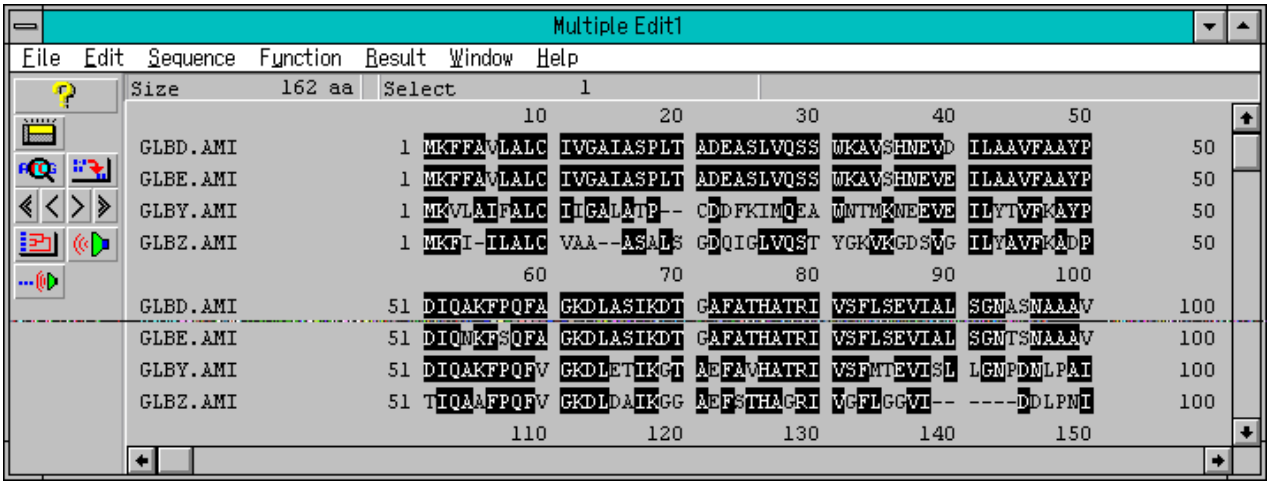
4. **Click OK to proceed with the alignment.**

   This opens a parameter dialog box. Here you may alter various search parameters.



5. **To start the alignment, click OK.**

   A progress bar will display the status of the alignment. The alignment window will open when the alignment it is completed.

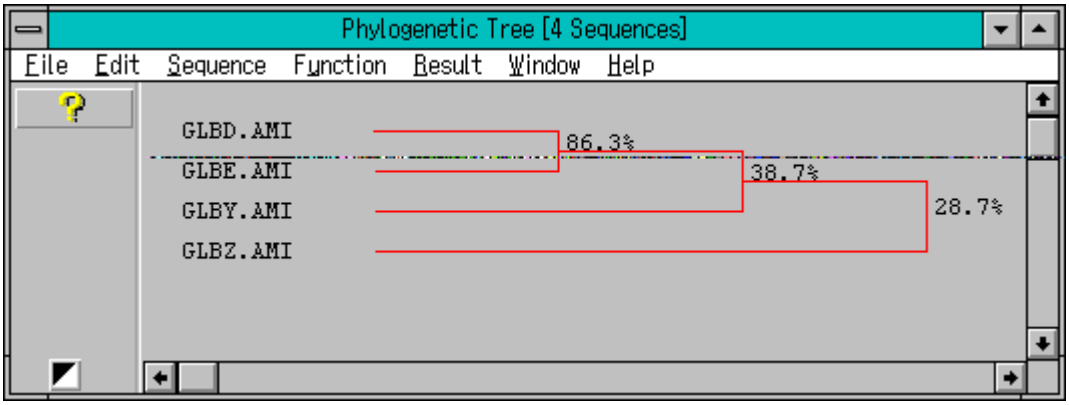


6. **You may use the keyboard or mouse to edit your alignment, e.g. insert or delete gaps.**

   As with the Contig Manager, you can save individual sequences by choosing Save or Save a Copy As. You can save the entire alignment by choosing Save List.

7. **Click  to display the Phylogenetic Tree.**

   This tree describes the percent similarities of the sequences in relation to each other.



You have now completed the demonstration for Multiple Sequence Alignments (Higgins). You can close these windows and continue with the demonstration or just exit the program.

Searching the BLAST Database

The Internet BLAST Search allows a similarity search to be performed against a standard sequence database. The BLAST algorithm is a heuristic for finding ungapped, locally optimal sequence alignments, and was developed by the National Center for Biotechnology Information at the National Library of Medicine. The BLAST family of programs employs this algorithm to compare an amino acid query sequence against a protein sequence database or a nucleotide query sequence against a nucleotide sequence database, as well as other combinations of protein and nucleic acid.

When preparing to submit a search, you must either save the sequence of interest in FASTA format or open it within DNASIS, which will automatically convert it to FASTA format.

A sequence in FASTA/Pearson format begins with a single-line description. The description line, which is required, is distinguished from the lines of sequence data that follow it by having a greater-than (">") symbol in the first column. In order to successfully pass through all computers that may need to relay the search request to the NCBI, all lines of the sequence (including the description line) should be kept to 80 characters or less in length. Example sequence in FASTA format:

```
>MNKSV40 Monkey DNA fragment of unknown function
ggttaaaatggtgatttttatgctttgtgtatttaccacttttttttttttaaggcaga
ttcctttcaatcatctgagtgagcccagtgcgatctgaagggtccctacaggtggaagag
gcagtggccaggatcgcggt
```

Sequences are expected to be represented in the standard IUB/IUPAC amino acid and nucleic acid codes, with these exceptions: lower-case letters are accepted and are mapped into upper-case; a single hyphen or dash can be used to represent a gap of indeterminate length; and in amino acid sequences, U and * are acceptable letters as Selenocysteine and translation stop. Before submitting a request, any numerical digits in the query sequence should either be removed or replaced by appropriate letter codes (e.g., N for unknown nucleic acid residue or X for unknown amino acid residue).

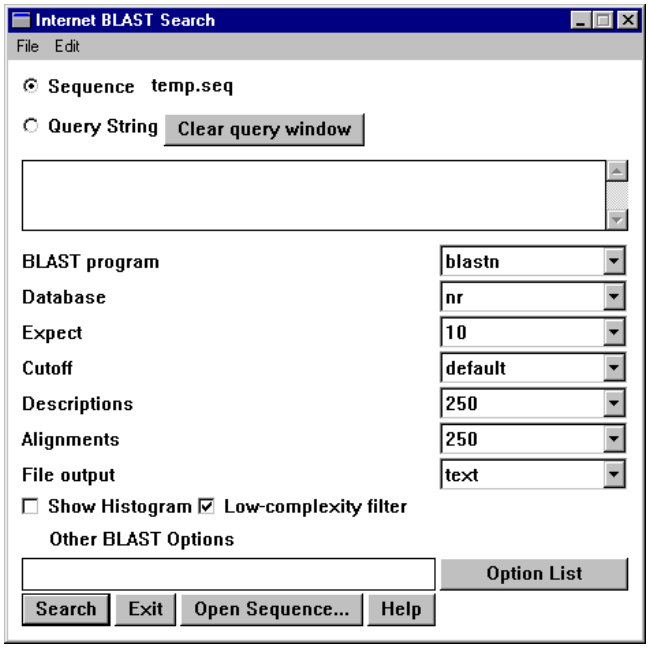
1. **Select File, Open and choose the AIDS1.DNA file from the Sample folder.**

2. **From the File menu, choose Open. Find and double-click on the folder called Sample Data and select the sequence named aids1.dna. Click OK.**

If a sequence is open in DNASIS, it will be saved to a temporary file (temp.seq) in FASTA format when the Internet BLAST search is invoked.

3. **From the Function menu, select Internet BLAST search.**

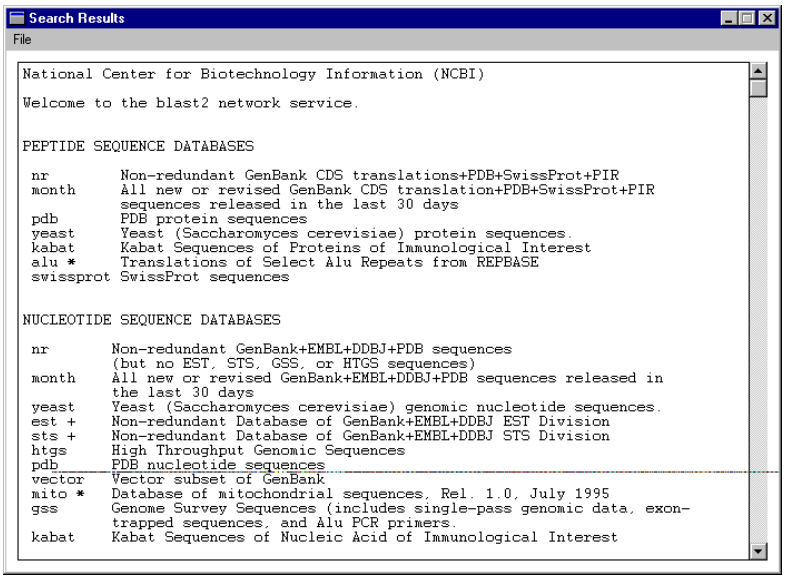
A dialog box appears and you may set the parameters for the search. First, make sure that the current DNASIS sequence (aids1.dna) is selected by checking the button next to Sequence temp.seq.



4. **Select the blastn program and the nr Database. Set the Expect value for 10, Cutoff for default, Descriptions and Alignments for 50, and the File output as text. Check the boxes next to Show Histogram and Low-complexity filter.**

5. **Click Search.**

You will see a status message from the BLAST server indicating if there are other searches pending. When the search is complete, you may see a message indicating that the complete list is not shown due to your parameter settings. Click OK. A window will appear with your search results.



You have now completed the demonstration for Internet BLAST search. You can close these windows and continue with the demonstration or exit the program.

Analyzing a Protein sequence.

In this portion of the Demo, we will select a DNA sequence, search for open reading frames (ORF), and then select and translate the ORF sequence. The protein sequence will be analyzed for hydrophobicity, secondary structure and isoelectric point.

1. **From the File menu, choose Open.**

2. **Find and double-click on the folder called Sample Data.**

3. **Select the sequence named pbr322.dna and click OK.**

4. **From the Function menu, go to Search and select Open Reading Frame.**

   This will open a dialog box. From here, you can set various options and parameters. The window also tells you which codon preference table is being used. You may change the codon usage table in Parameter Table under the Function menu.

5. **Click the Go button.**

   After the analysis is complete, an ORF map window will pop up. The graphics displayed in this window show the predicted ORFs in each reading frame. Initiation codons are shown as triangles and termination codons are shown as verticle lines across the sequence.

6. **Move the mouse down to the Frame 2 column and click to the right of the first start codon (red triangle).**

   This will select the ORF of the second reading frame from base 86 to 1276.

7. **Go to the side menu bar and click the**  **translation button.**

   You will now see the predicted protein sequence of that open reading frame. Leave this window open.

8. **Close the PBR322.DNA edit window and the ORFs map window.**

We have now analyzed a DNA sequence for an open reading frame; we have also selected and created a protein sequence using the ORF analysis. In the next step, we will analyze the protein sequence for hydrophobicity and protein secondary structure.

Analyzing a Protein Sequence

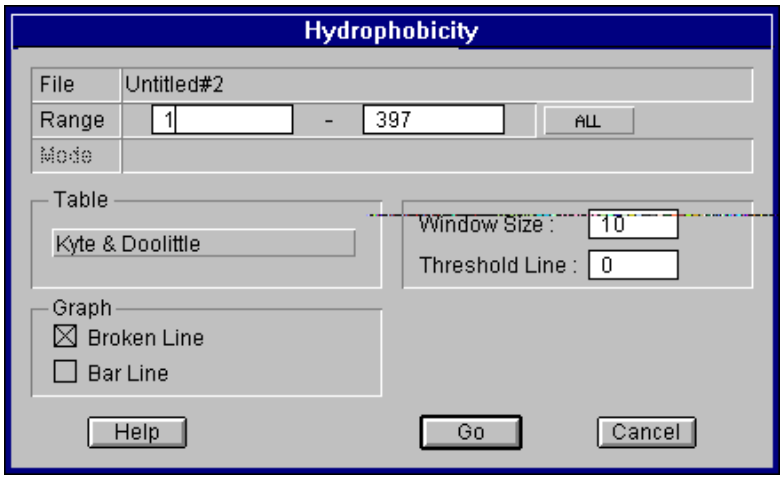
- Hydropathy Plot
- Protein Secondary Structure
- Protein Helical Wheel
- Isoelectric Point Calculation

9. On the side menu bar of the DNA Translation window, select th editor button.

This will open an edit window of the protein sequence.

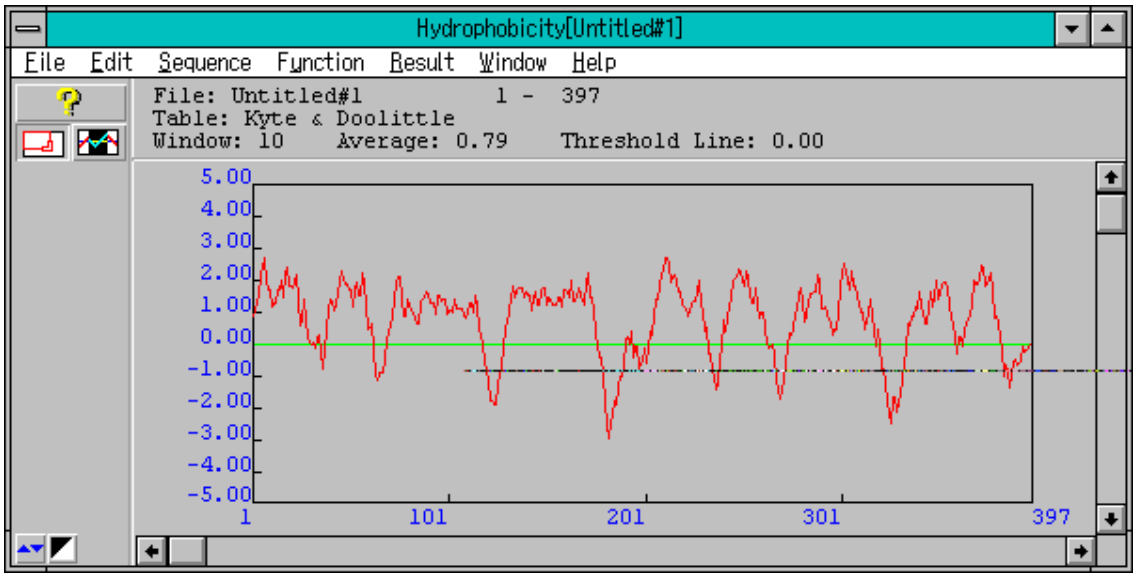
10. From the Function menu, go to Search and select Hydrophobicity.

This will open a dialog box. From here, you may set the algorithm and other parameters. Select method of analysis by choosing Kyte & Doolittle next to Table:. Set the window to 10 and the Threshold to 0.



11. Click the Go button.

After the analysis is complete, the Hydrophobicity plot will be displayed.



Altering the Display

The side menu bar of the Hydropathy window allows the user to change the display.

- With the  button, you can enlarge the plot by dragging the lower right corner of the plot display box with the mouse.

- By selecting the  button, you can zoom in on specific regions of the plot. Use the mouse to select a portion of the plot. This will zoom in the selected area. Double clicking on the plot will zoom out.

11. Close the window after you are done viewing.

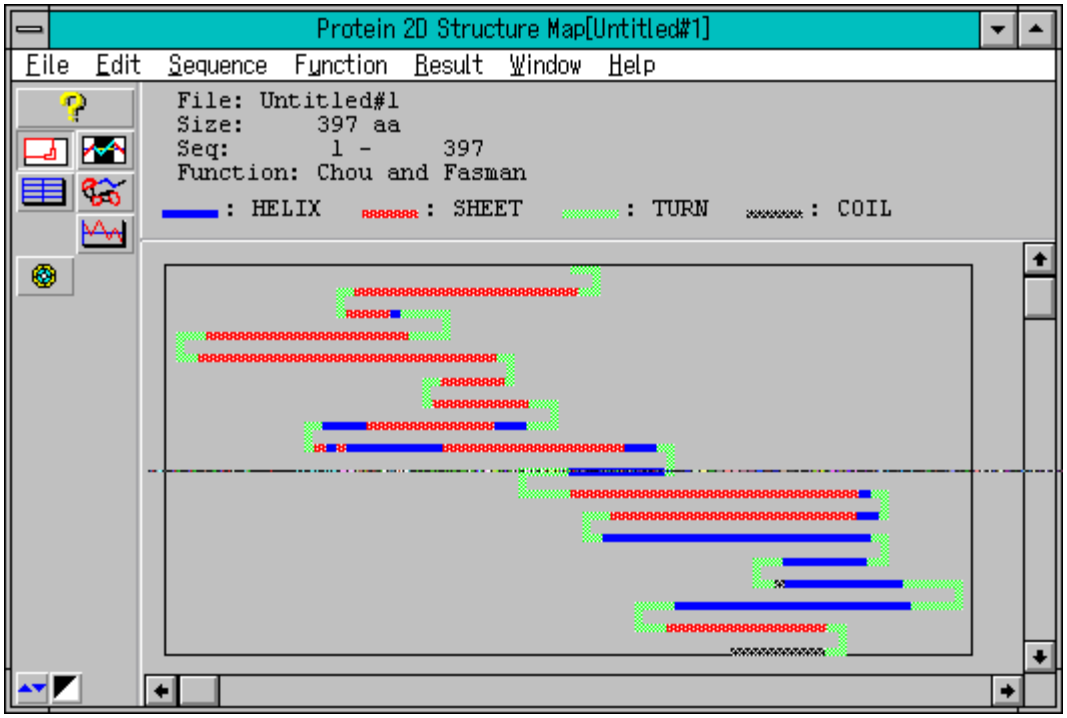
12. Under the Function menu, go to Prediction and select Protein Secondary Structure.

13. A dialog box will allow you to choose different analysis methods; select Chou and Fasman.

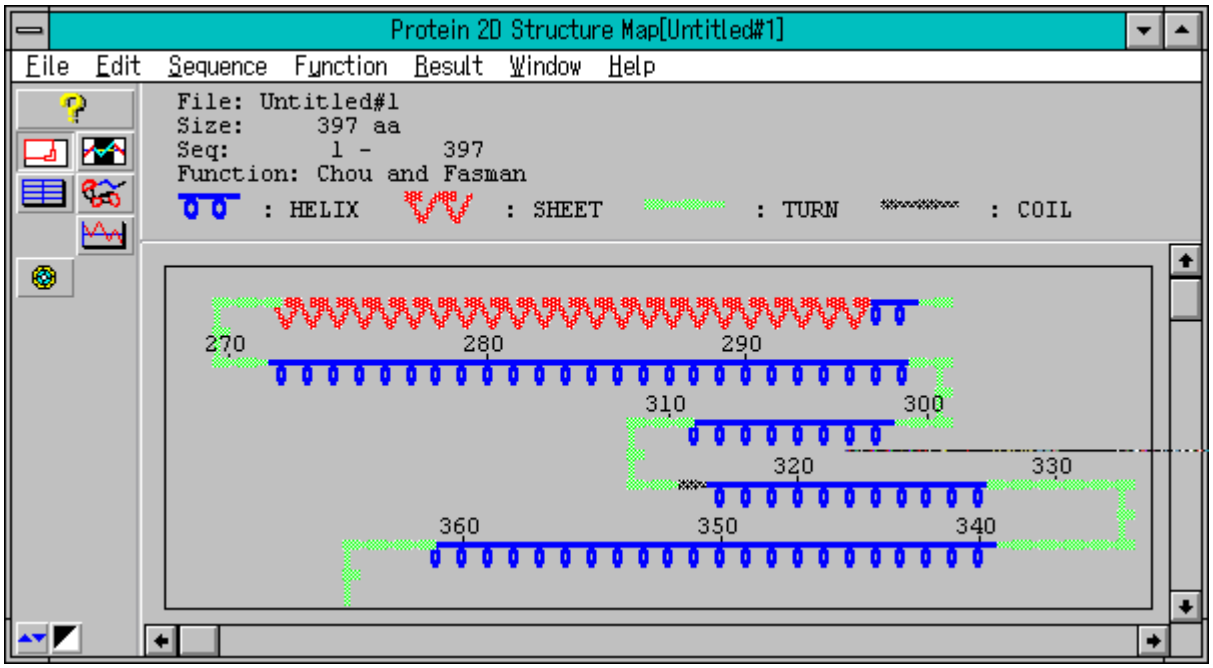
This will display a Protein 2D Structure Table window.

14. Click on the map display button  .

A window will open representing the protein secondary structure in a graphical map format. You can alter the display here the same way you did with the hydrophobicity plot.

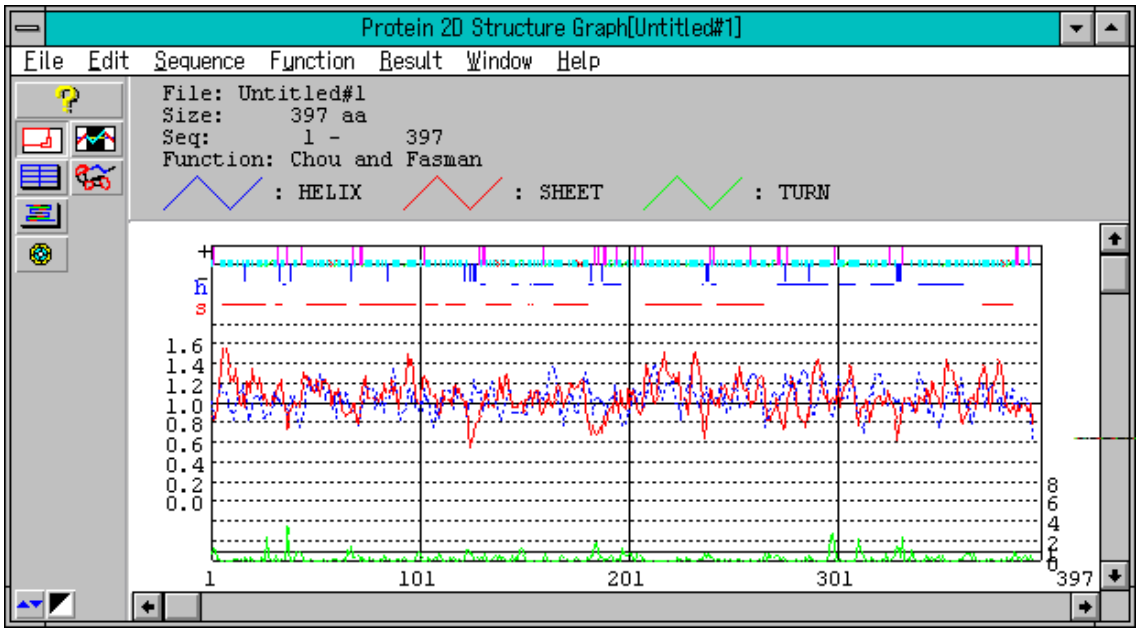


15. **Select the image expand icon  . With the mouse, select a small region of the display. The image will automatically expand. To return image to normal size, double click the image.**



16. **To display the data in graph form, selec  graph icon.**

The calculated values for the three conformational conditions are plotted against residue number. The helix, and sheet traces are superimposed on one another. The beta-turn trace is plotted separately in green at the bottom of the graph.

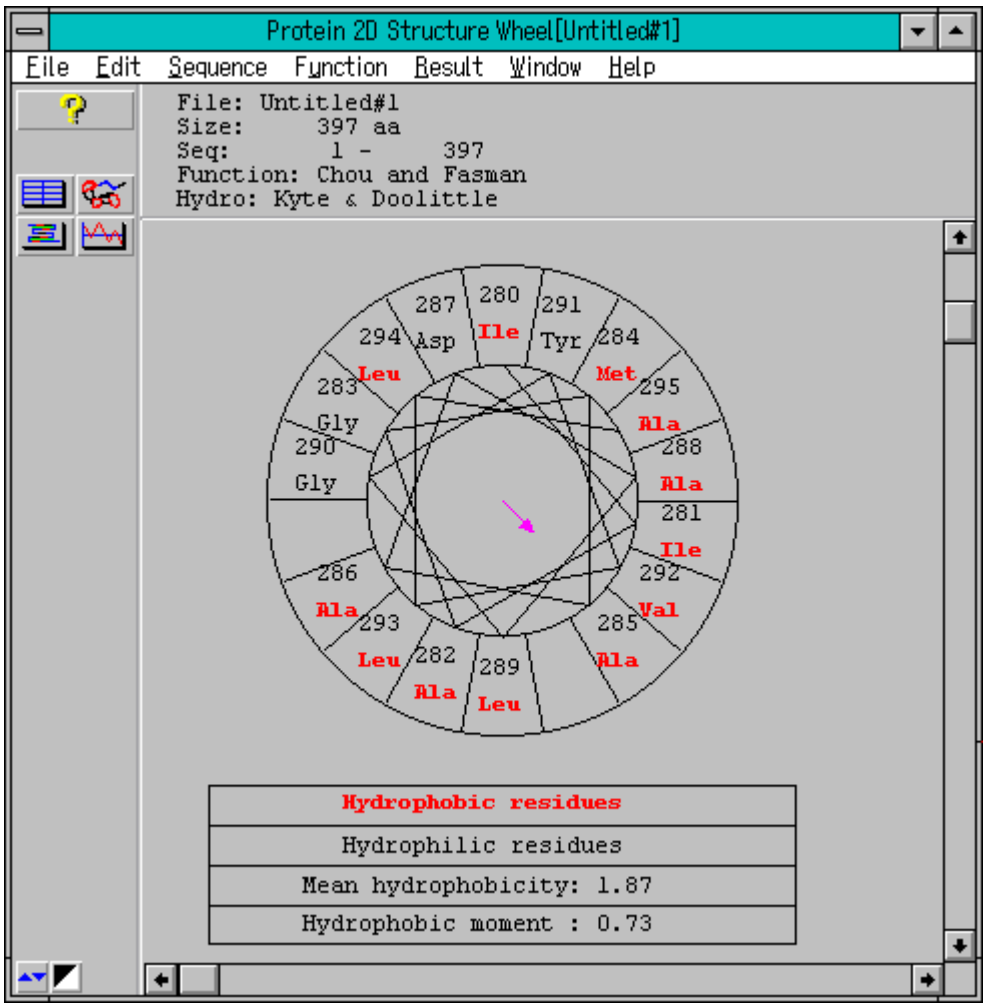


You can display this in a linear format by clicking on the  button.

Helical Wheel Display

17. Click the helical wheel display button, select the range 280 to 295 and click Go.

This will create a helical wheel representation of residues 280 to 295.



Helical Wheel representation of residues 280 to 295, alpha helix prediction from Chou and Fasman.

18. When you are done, close this window.

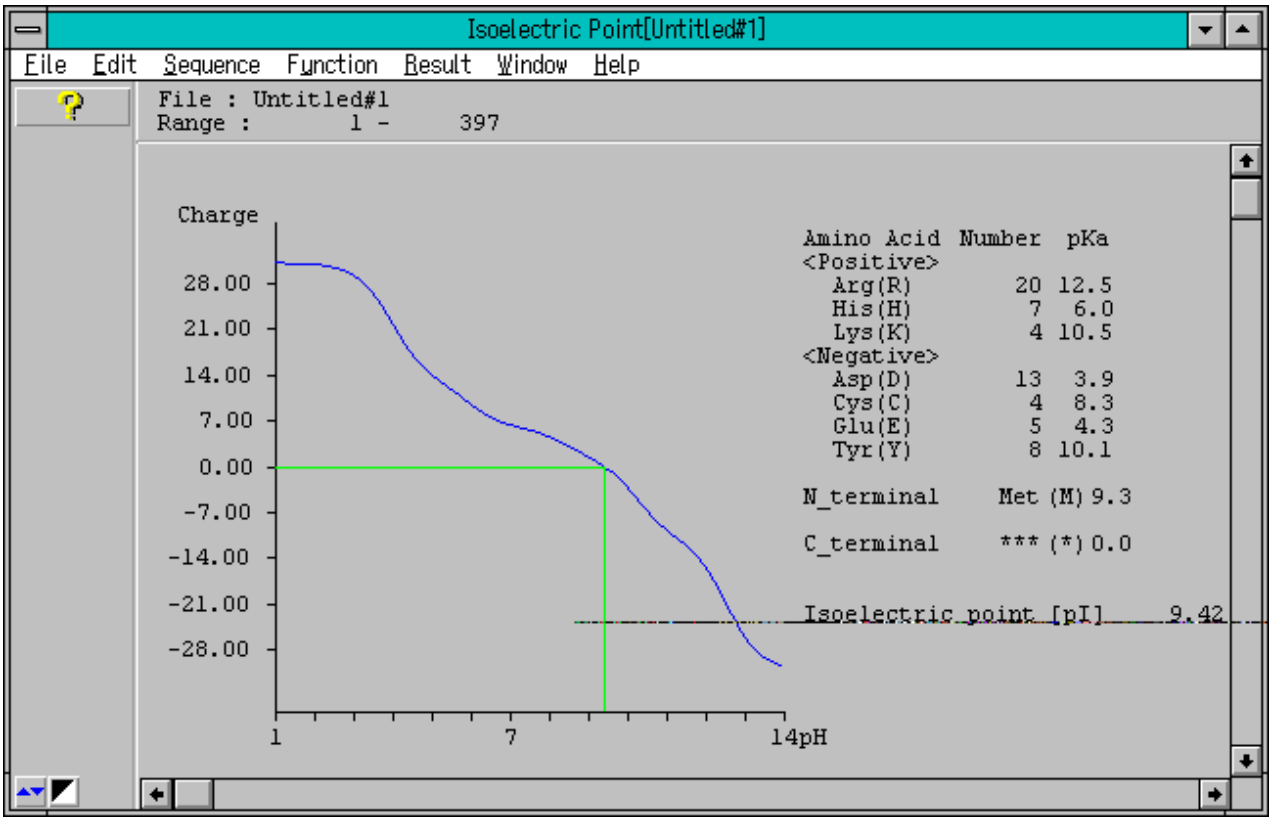
You have now completed the demonstration for Analyzing a protein sequence. You can close these windows and continue to explore DNASIS or quit the program.

Predicting the Isoelectric Point of a Protein.

19. From the Function menu, go to Prediction and select Isoelectric Point.

This will open a dialog box; here you can set the range for the for the prediction.

20. Click Go. After the calculation is complete, a results window will open.



The graph describes the predicted pI of the selected protein. The positive and negative amino acids are also listed in the table.

You have now completed the demonstration for Analyzing a protein sequence. You can close these windows and continue to explore DNASIS or quit the program.

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