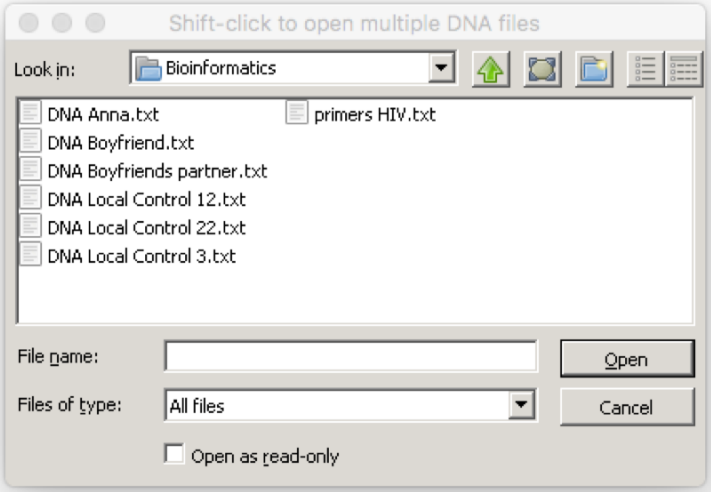
Tutorial for using Case It for bioinformatics analyses

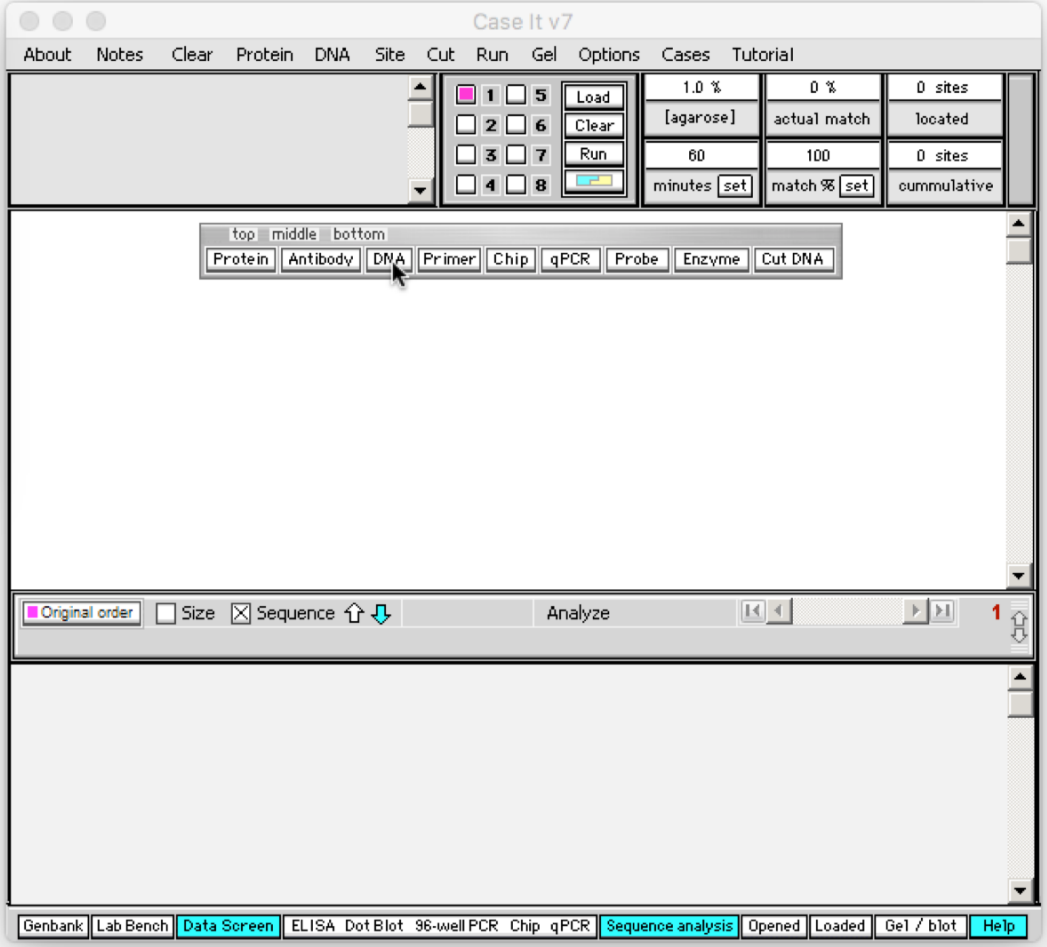
Preparation of sequences for multiple alignment and tree-building, using

- the MABL web site,
- the MAFFT web site, or
- MEGA5 bioinformatics software

Blasting DNA and protein sequences

Open Case It v7.exe to the Data Screen, then Click the **DNA** button on the silver button bar. For this example, we have opened **Case It -> Infectious Diseases -> HIV -> U.S.-> Anna-> Bioinformatics**. Note that there are files for Anna, her boyfriend, the boyfriend's partner, and three local controls, along with a primers file.





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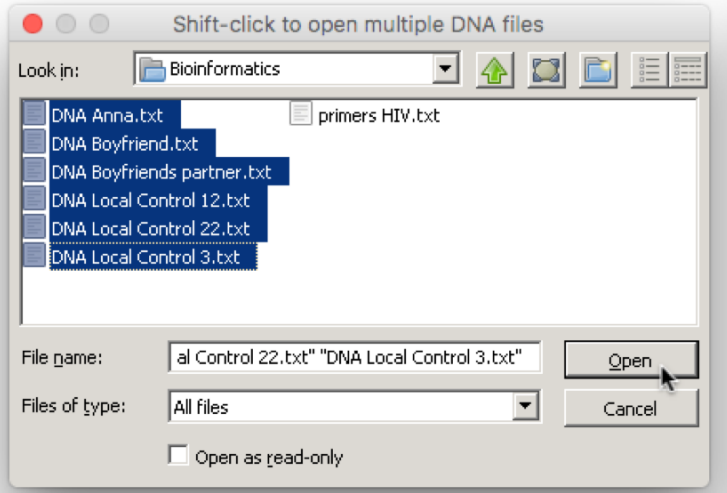
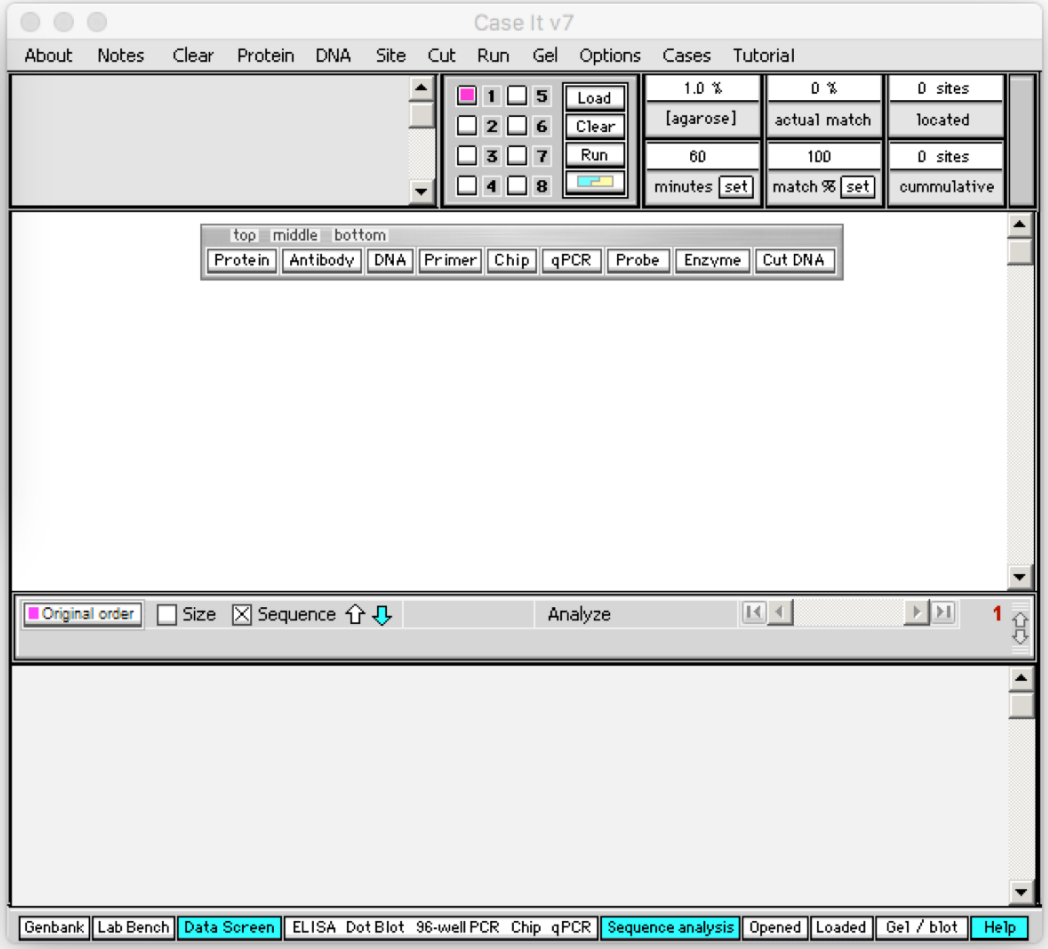
Click on the first DNA file you want to open [**DNA Anna.txt** in this example], hold down the Shift key, and click on the last DNA file you want to open [e.g. **DNA Local Control 3.txt**]. Then click the **Open** button, or double-click on the last file while still holding down the **Shift** key. Be careful not to include the primers file along with the DNA files.





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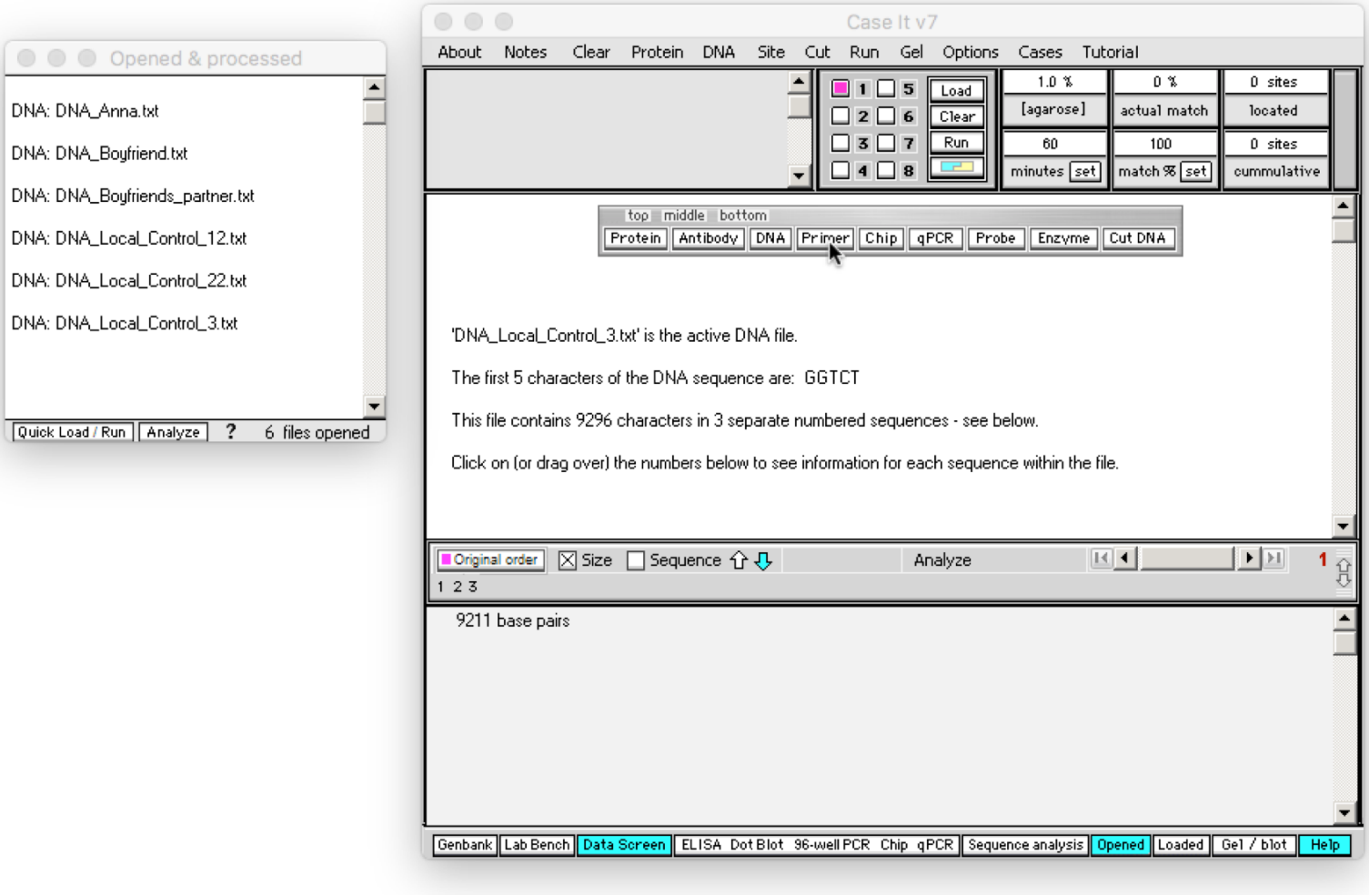
The **Opened & Processed** window opens, with each file designated by a line in this window. For this particular example, we need to work with PCR products, rather than the original files, to make the sequences shorter [this step may not be necessary, depending on the case being analyzed]. To begin the PCR process, click the **Primer** button on the silver button bar…



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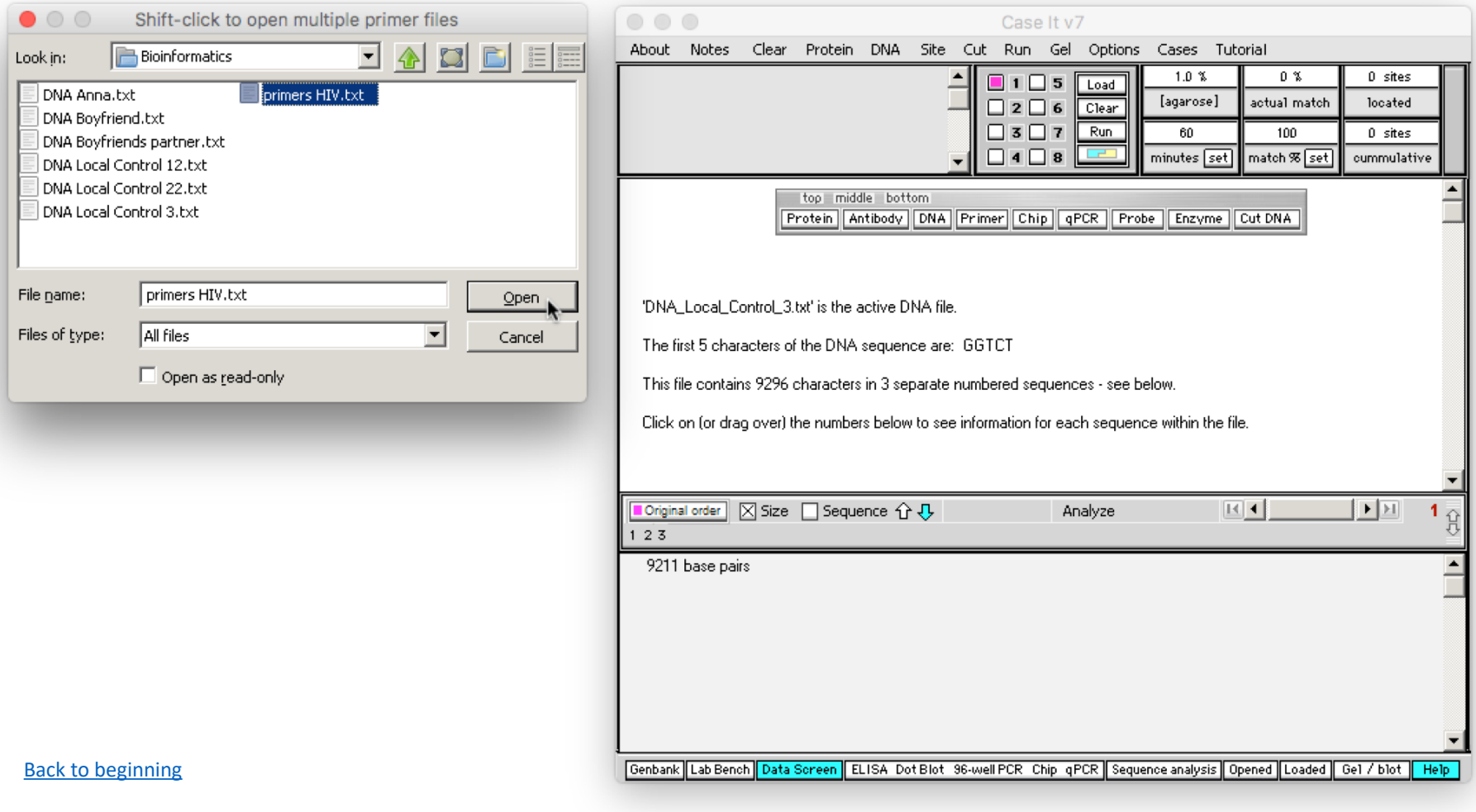
…then select the primers file and click **Open** [or double-click the file name **primers HIV.txt**]…



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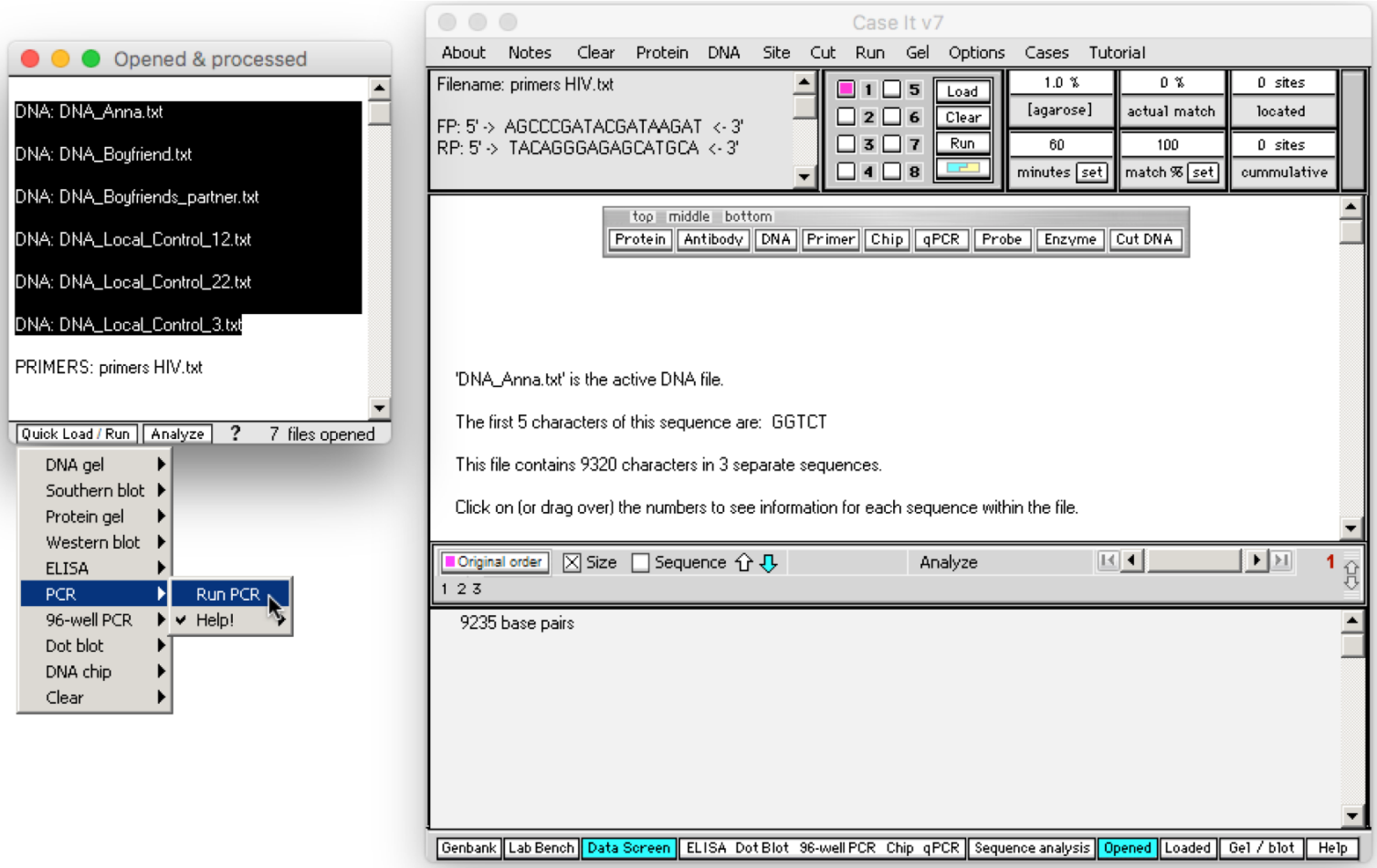
The primers file name appears in the **Opened & Processed** window, and the forward and reverse primers of this file appear, indicating that this primer file is active. **Shift-click** to highlight the DNA files, then use the **Quick Load /Run** button to select **PCR -> Run PCR**.



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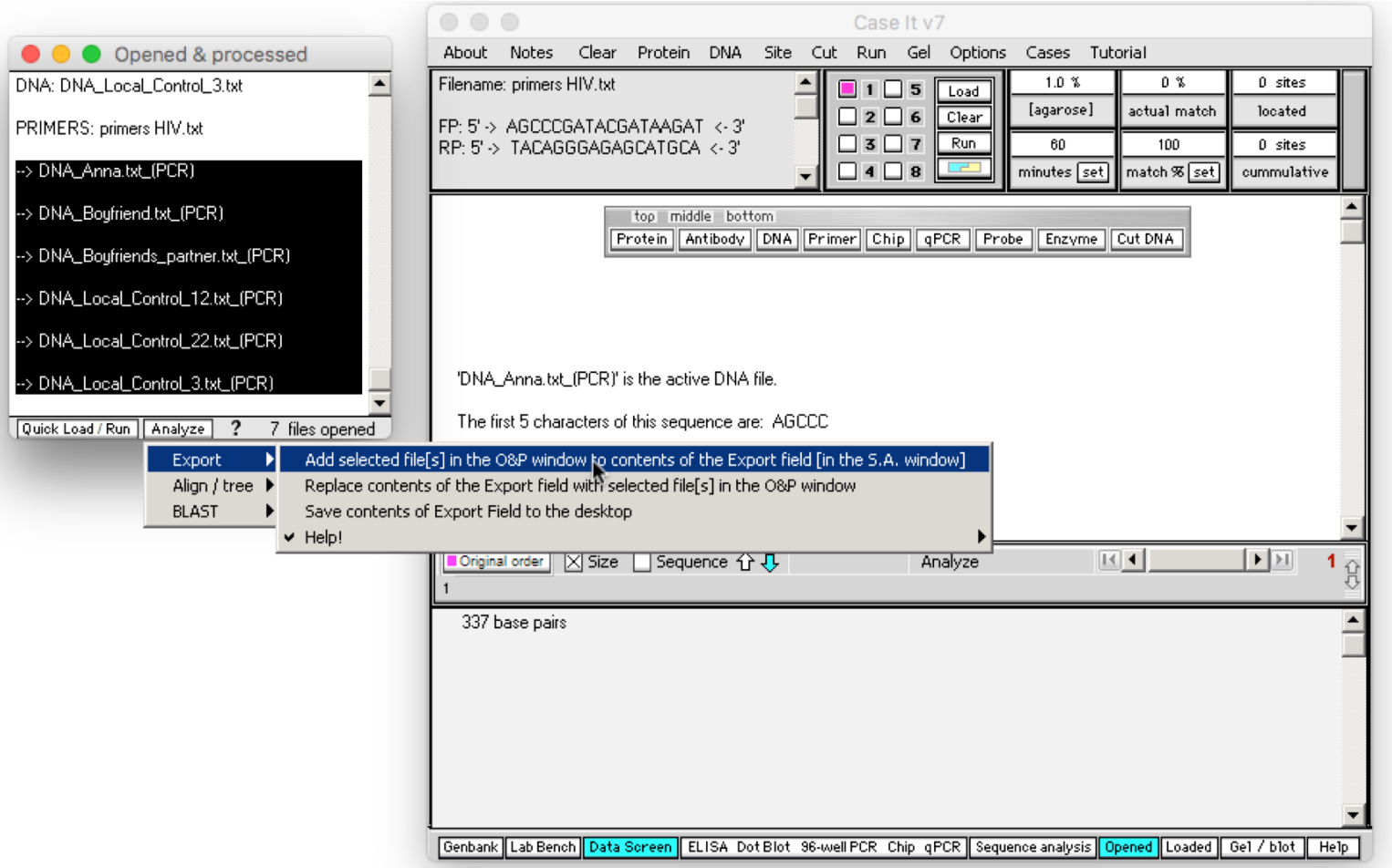
New names appear in the Opened and Processed window, with each name preceded by an arrow symbol. These names represent PCR products. **Shift-click** to highlight the names, then use the **Analyze** button and select **Export -> Add selected file[s] in the O&P window to contents of the Export field [in the S.A. window]**.



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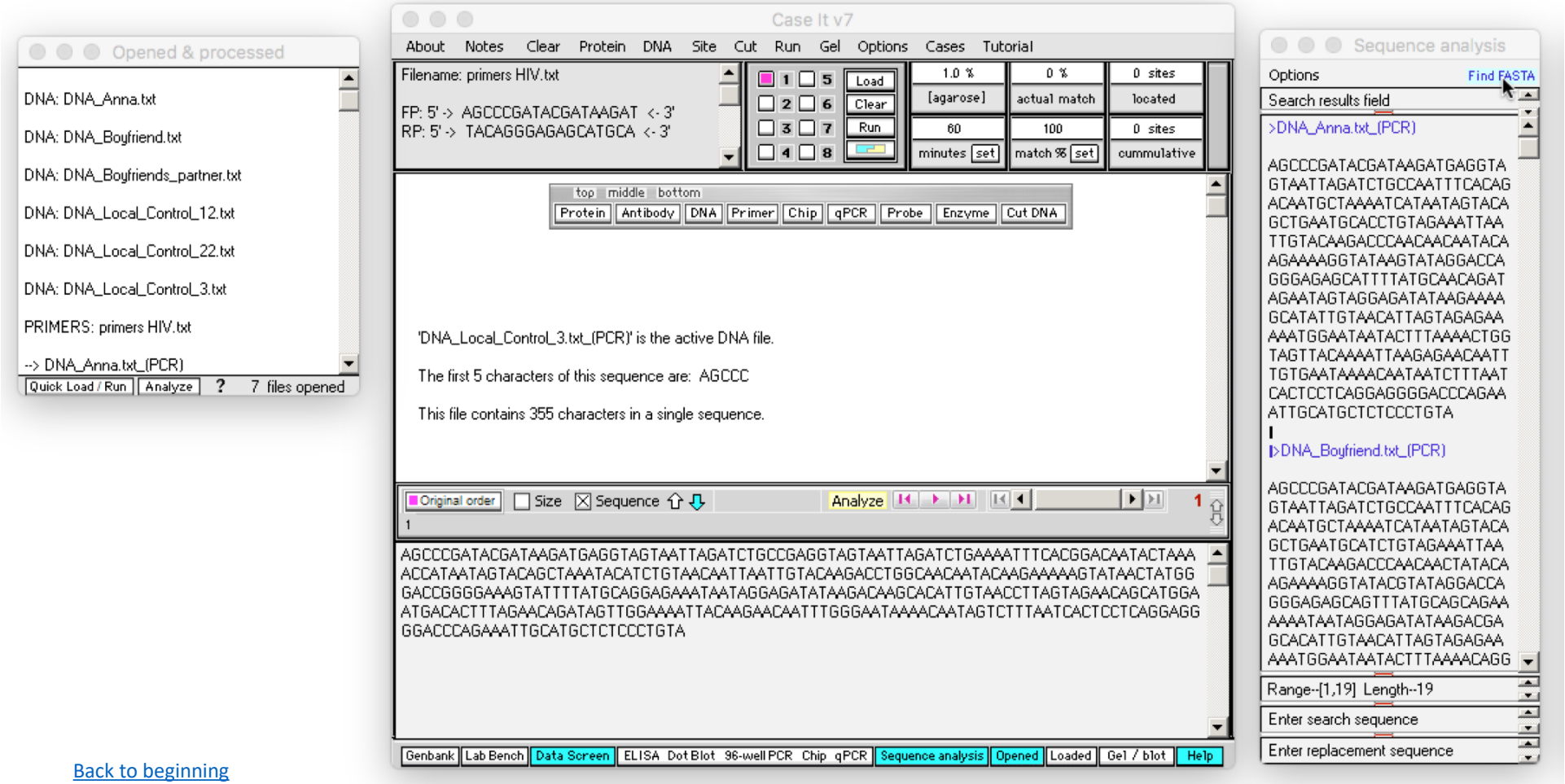
Selected PCR products have been added to the **Sequence analysis** window to the right of the main screen, in FASTA format. [Note that you can verify that the proper products were added by repeatedly clicking the blue **Find FASTA button** in the Sequence analysis window, to cycle through the products.]



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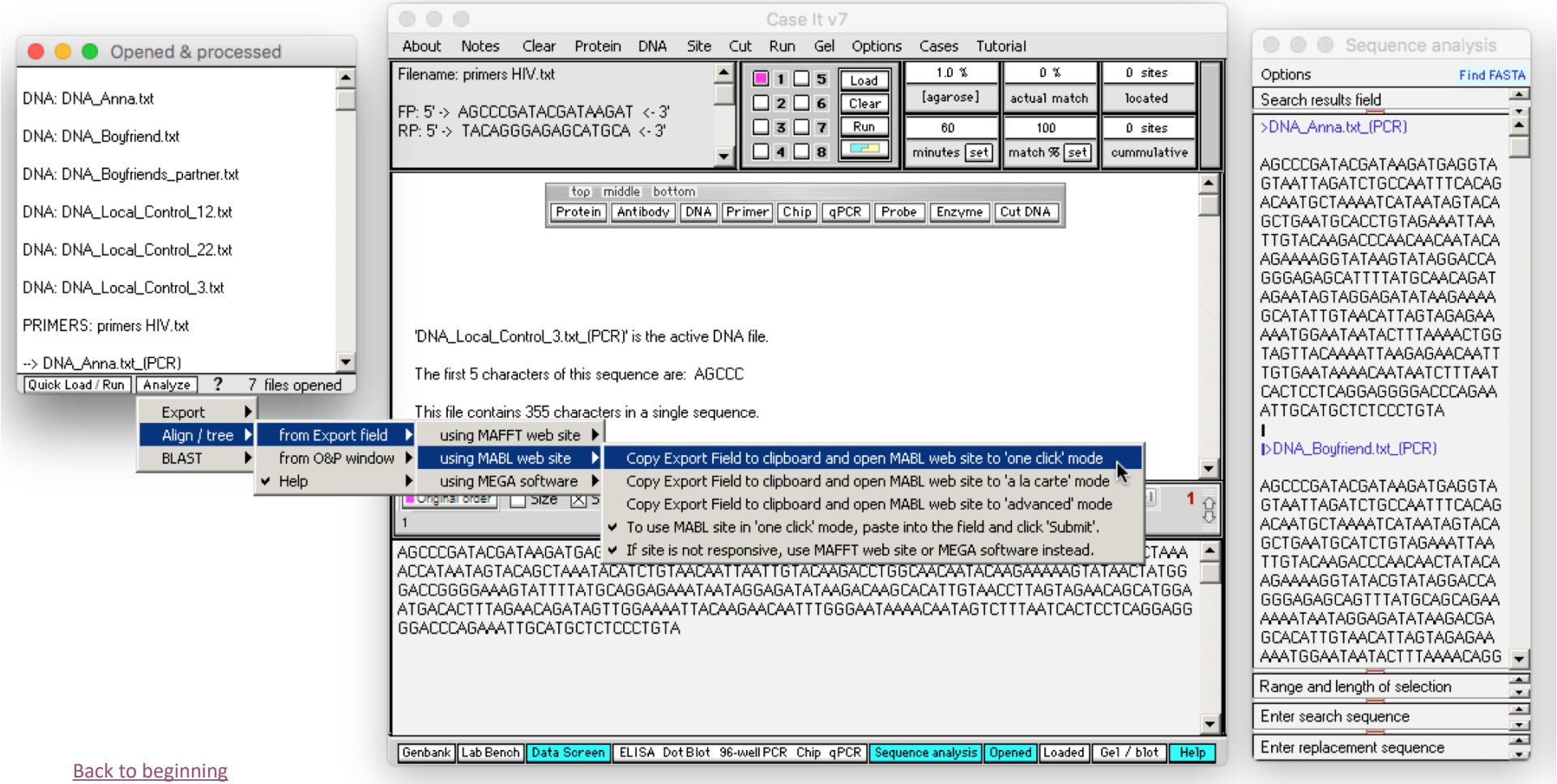
Case It has three options for aligning sequences and building trees [1] MABL web site, [2] MAFFT web site, and [3] MEGA software. The quickest way to build a tree is with the MABL, so we'll demonstrate that first. To use the MABL website, click the **Analyze** menu at the bottom of the Opened & processed window, and select the menu choices shown below. We'll use the 'one click' mode of MABL for simplicity.

[Note: the MABL website is not always responsive, so if it doesn't work it may be necessary to use MAFFT or MEGA instead.]



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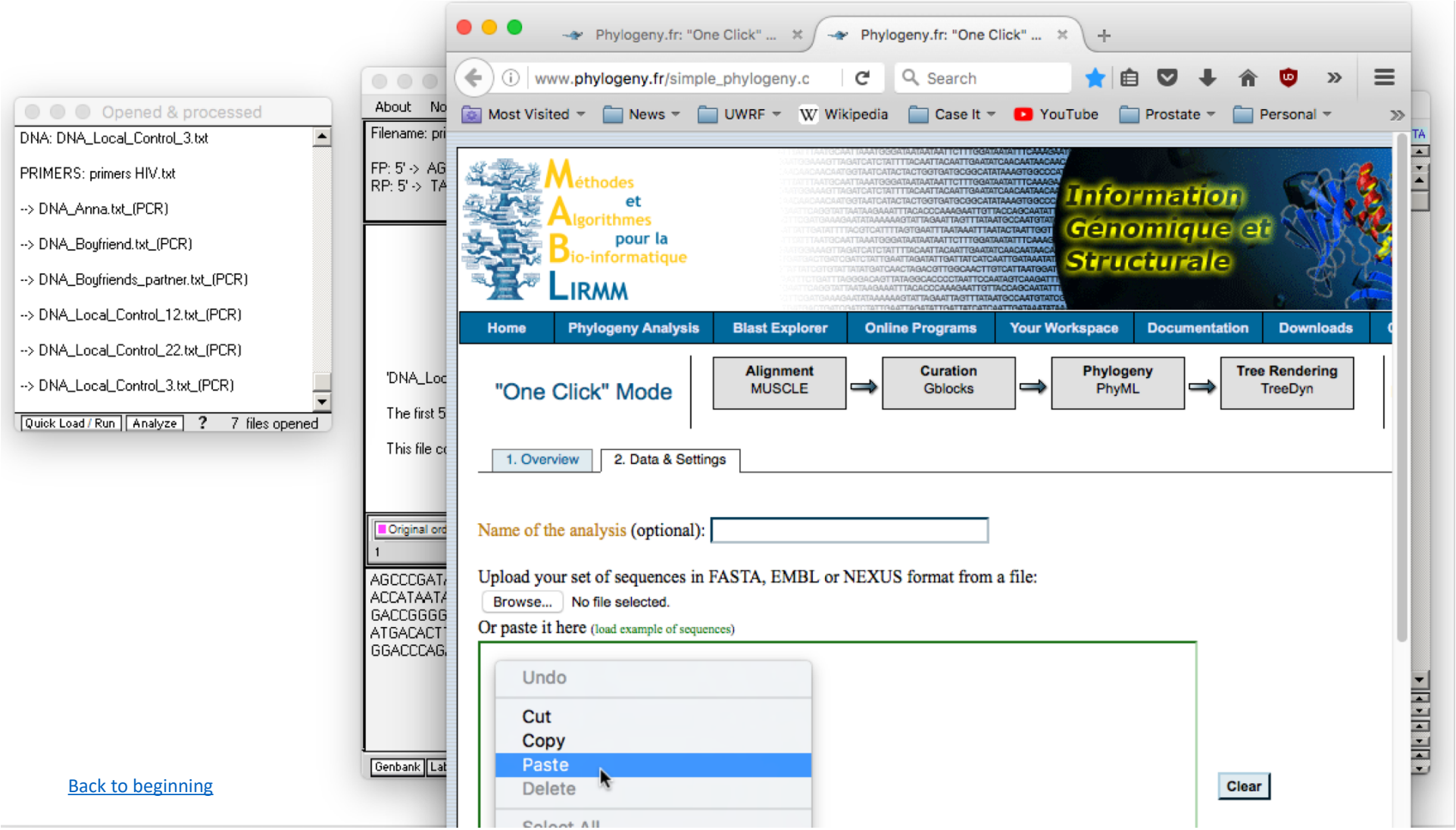
The MABL web site automatically opens to ‘once click’ mode. **Right-click** on the input field and select **Paste**…



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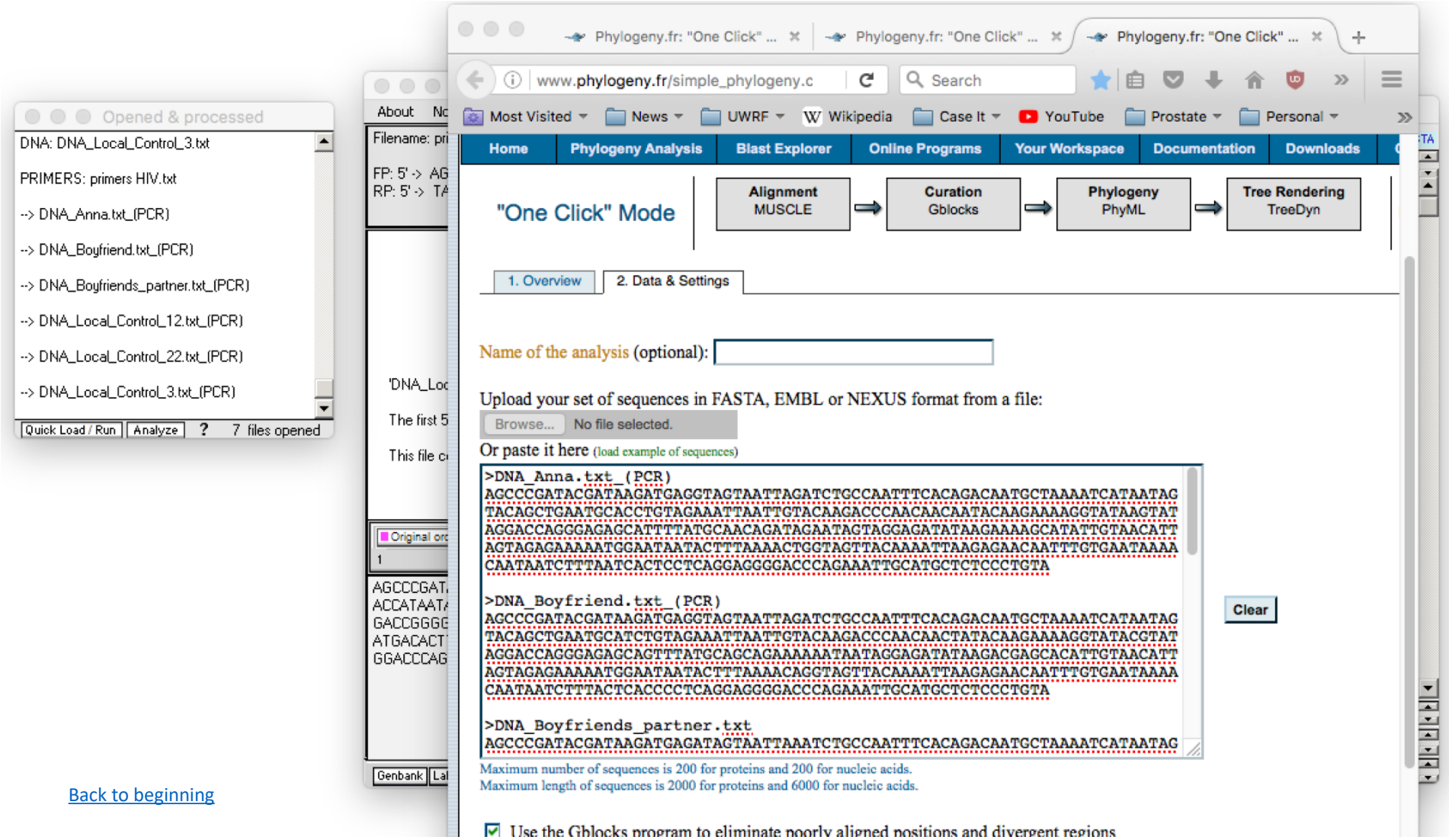
Contents of the Export field of Case It now appear in the input field of the MABL web site.



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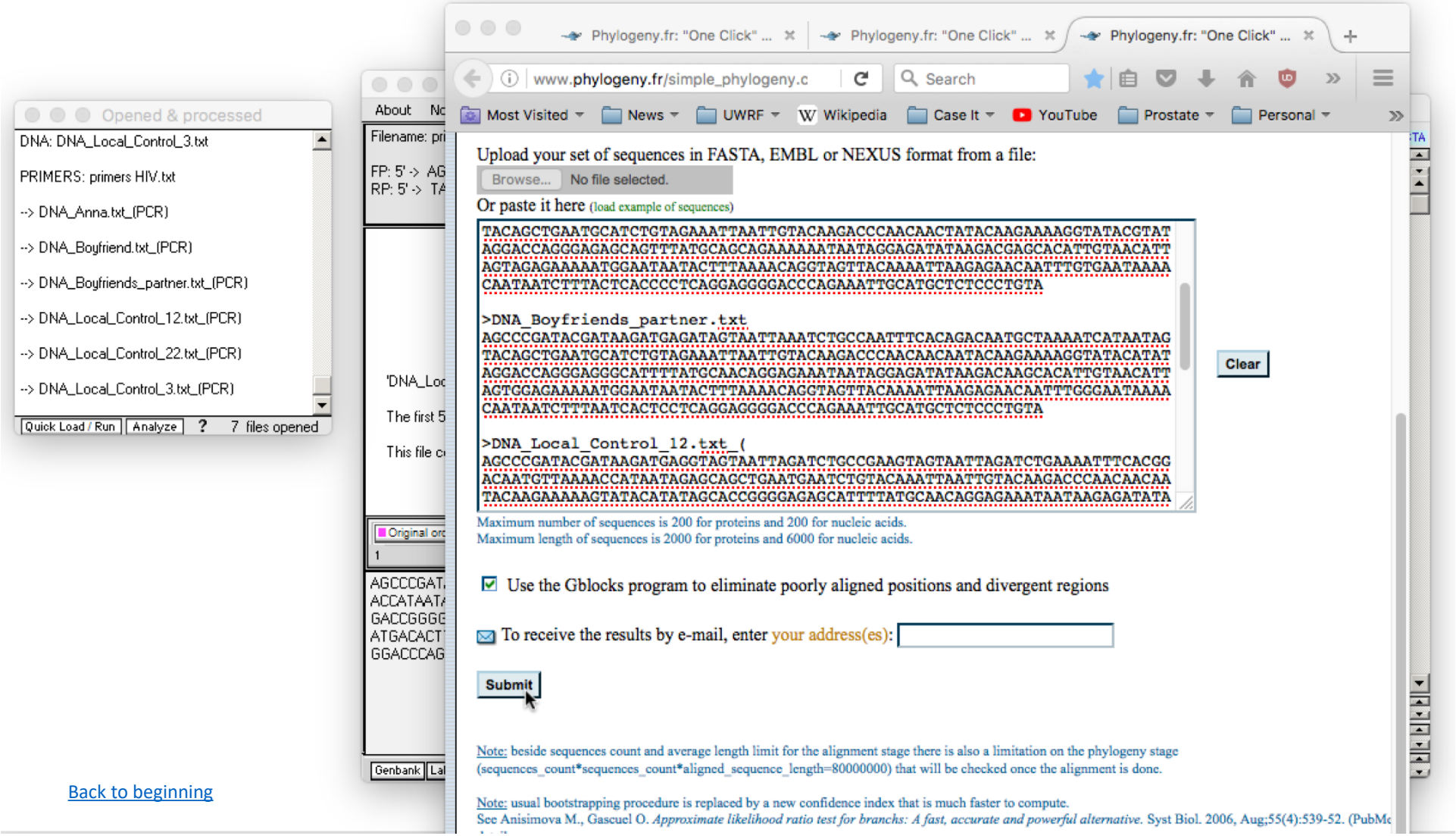
Scroll down on the web page, and click **Submit** (since this is the 'one click' mode of MABL)…



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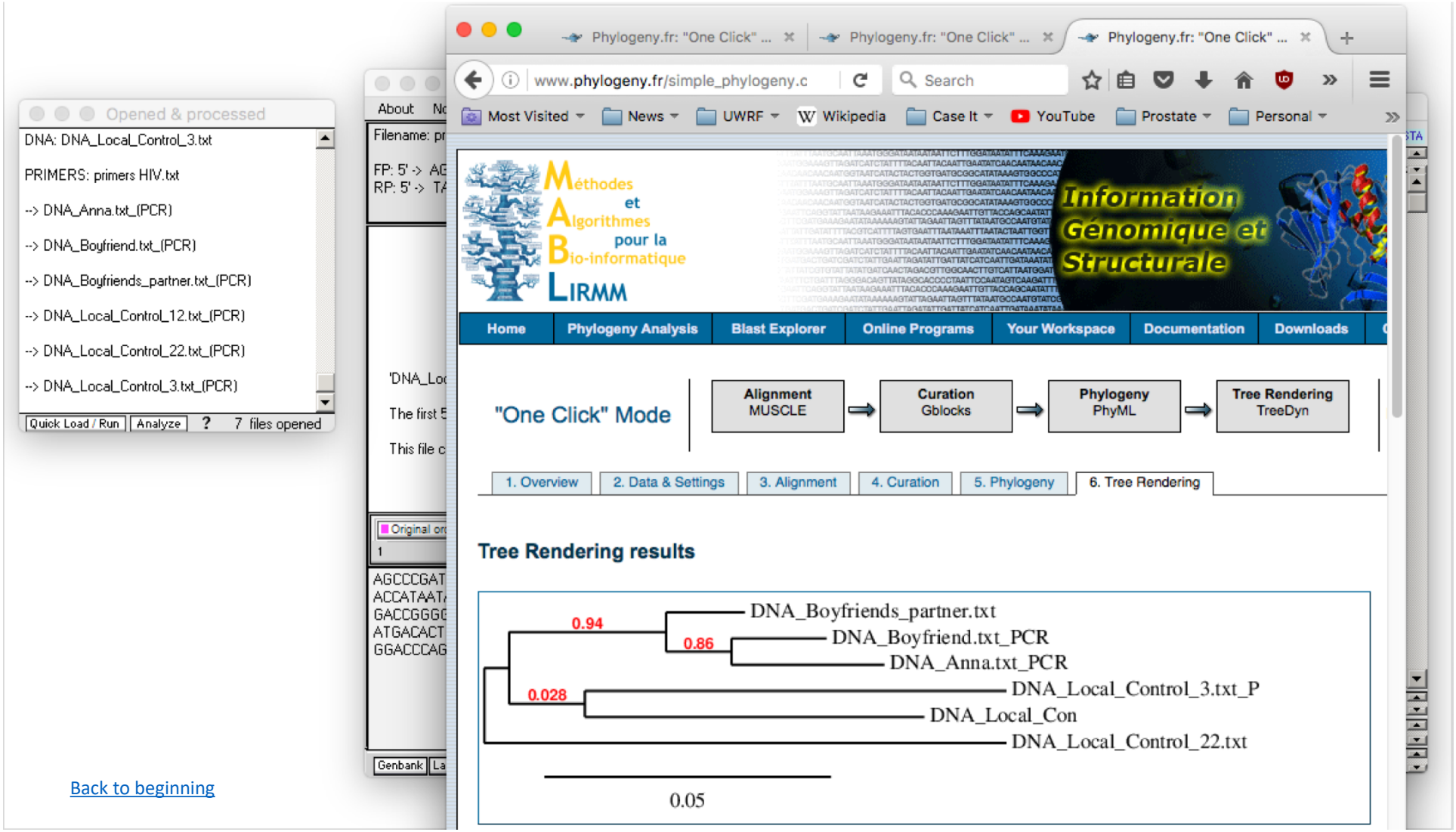
…and wait for the tree to appear. If the website is not responsive, use one of the other options [MAFFT web site, MEGA bioinformatics software].



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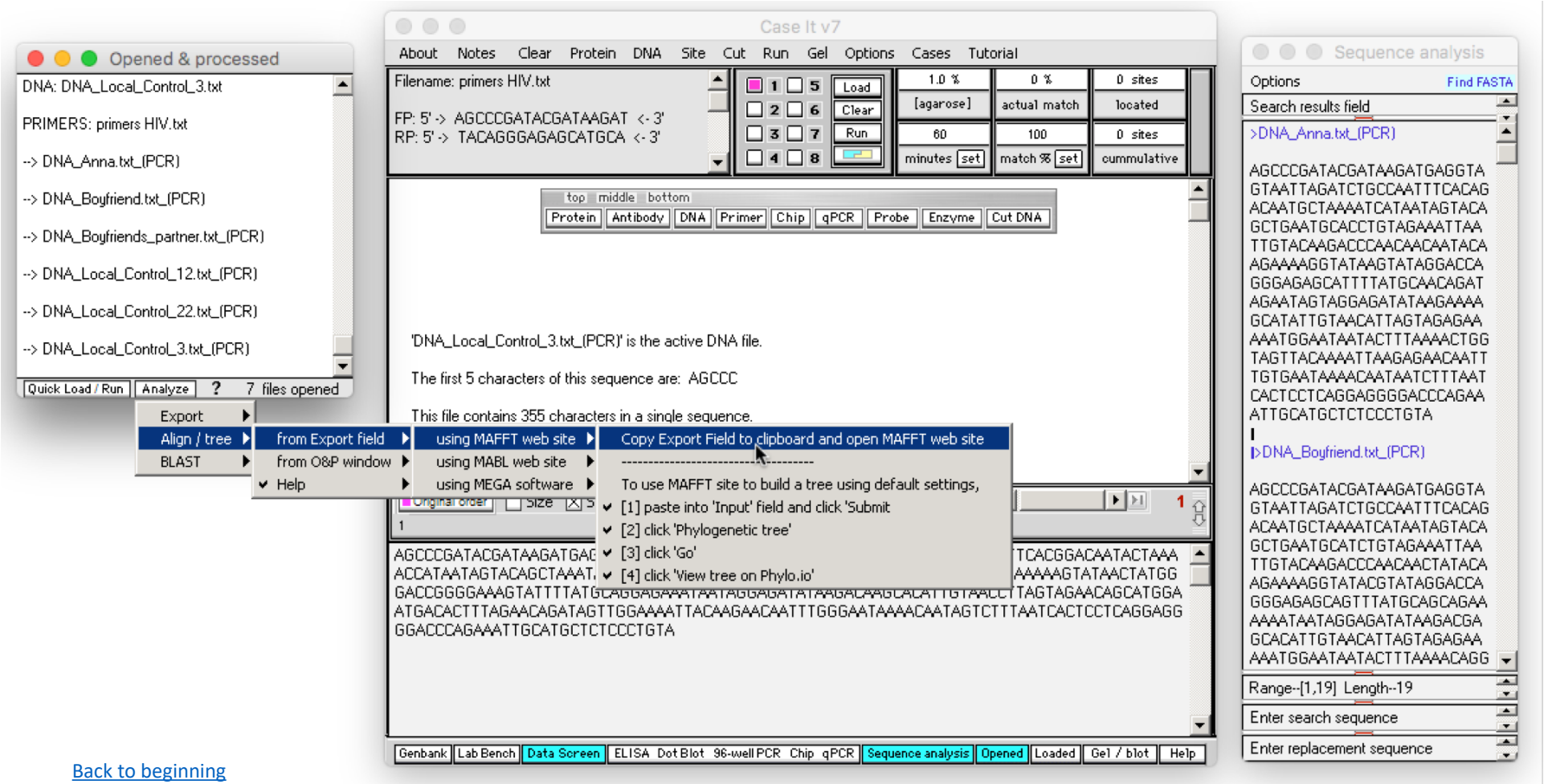
Case It has three options for aligning sequences and building trees [1] MABL web site, [2] MAFFT web site, and [3] MEGA software. Use the Analyze button and select the menu options shown below to copy contents of the Export Field to the clipboard and automatically open the MAFFT site. [Note: Although use of MAFFT requires more mouse clicks then using MABL, the MAFFT site is almost always operable, whereas MABL may not be.]



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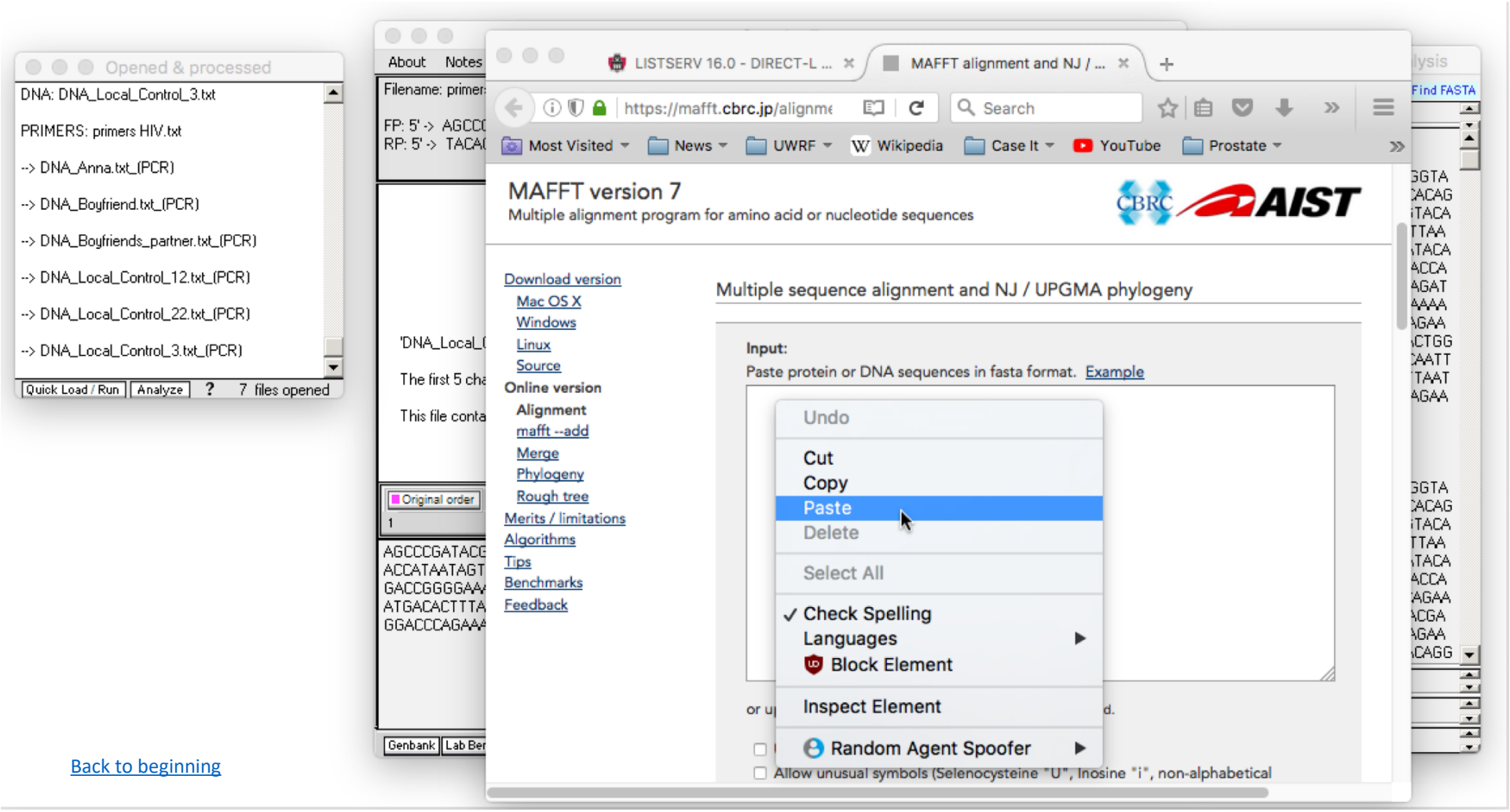
It may take a few seconds for your web browser to open to the MAFFT site. **Right-click** inside the Input field, and select **Paste**.



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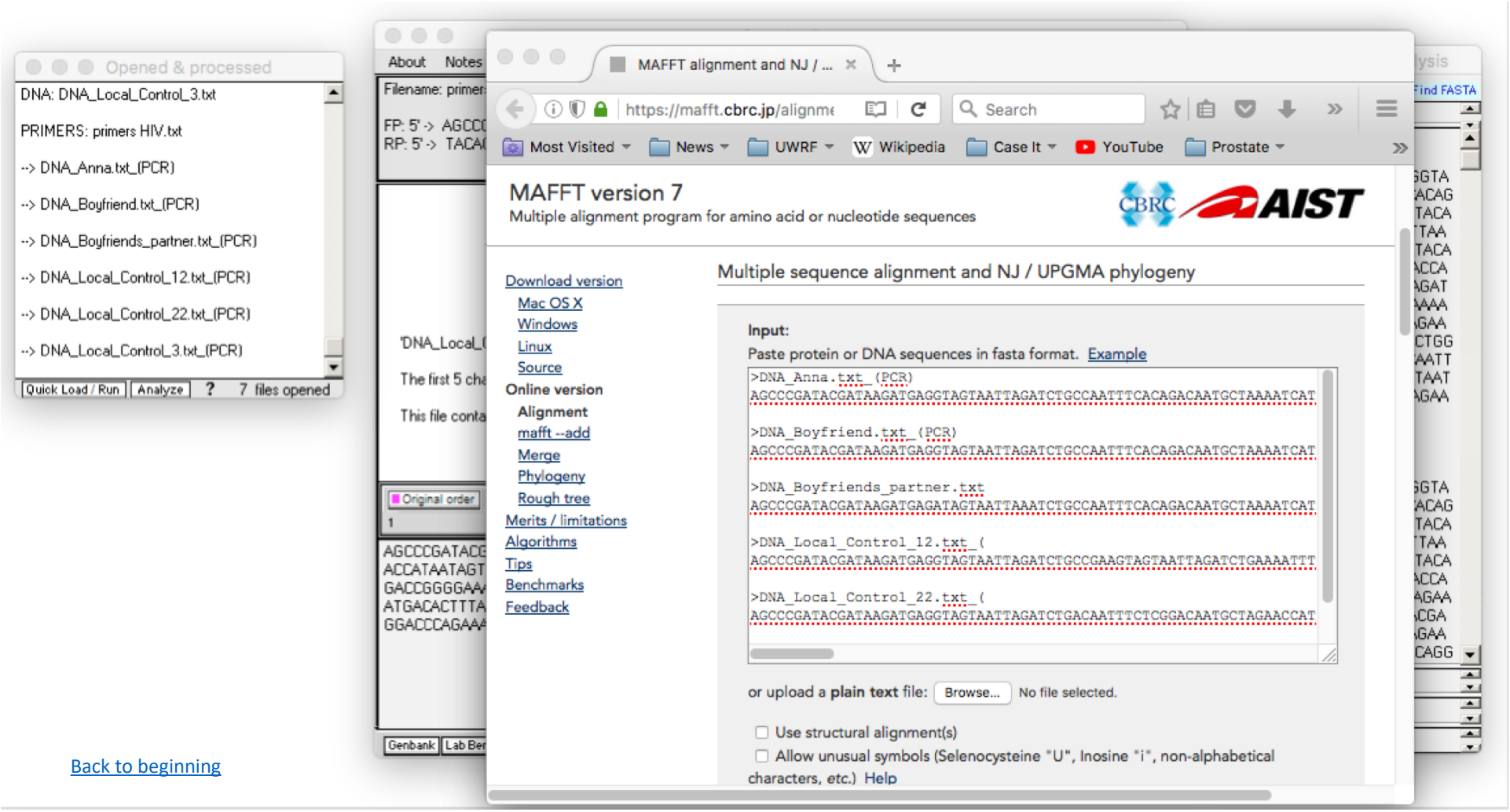
Contents of the Export field of Case It now appear in the Input field of the MAFFT web site.



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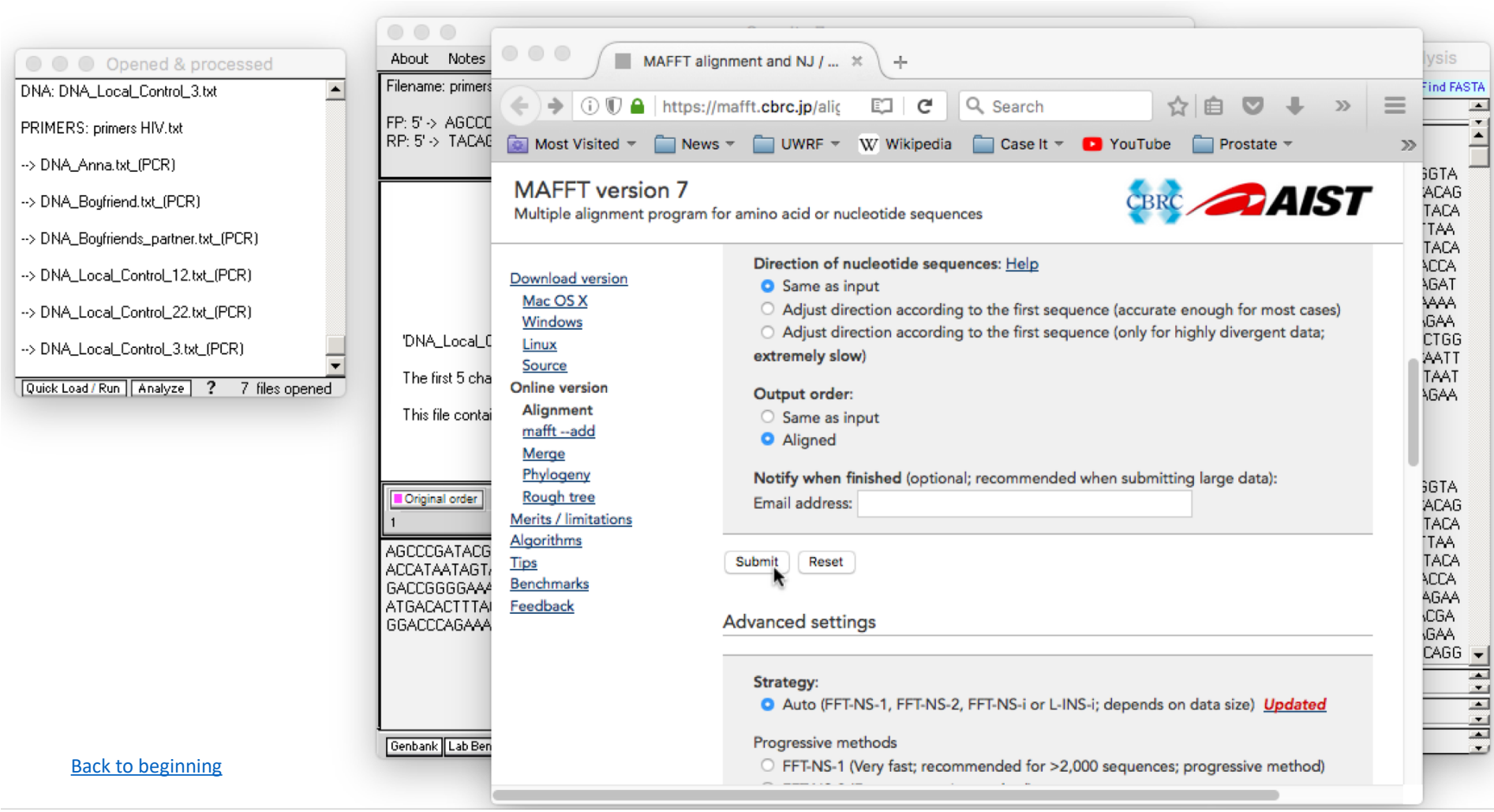
Scroll down on the MAFFT page and click the **Submit** button [in this example, no options are being changed before clicking Submit].



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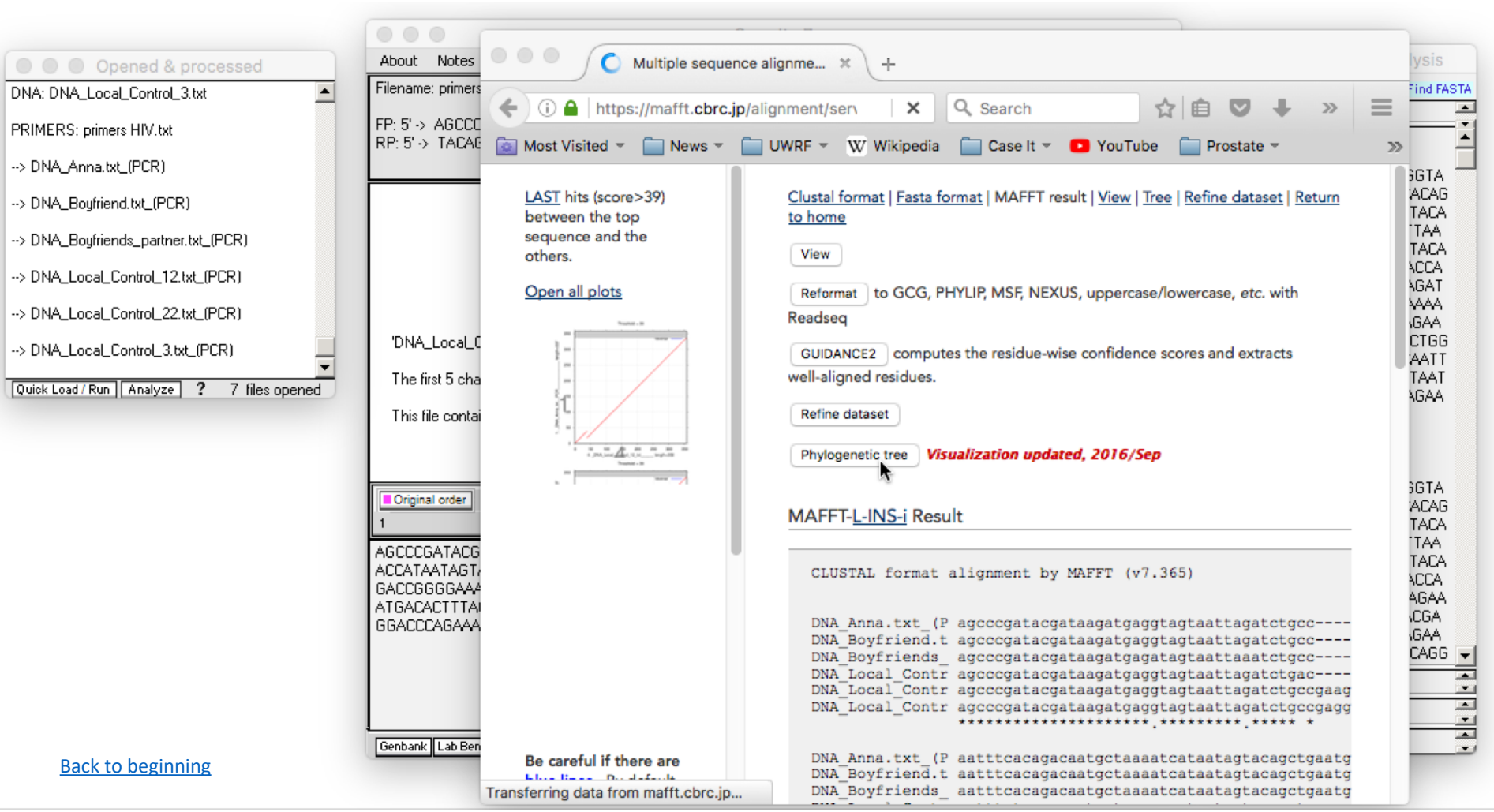
The sequences have been aligned via CLUSTAL. Click the **Phylogenetic tree** button…



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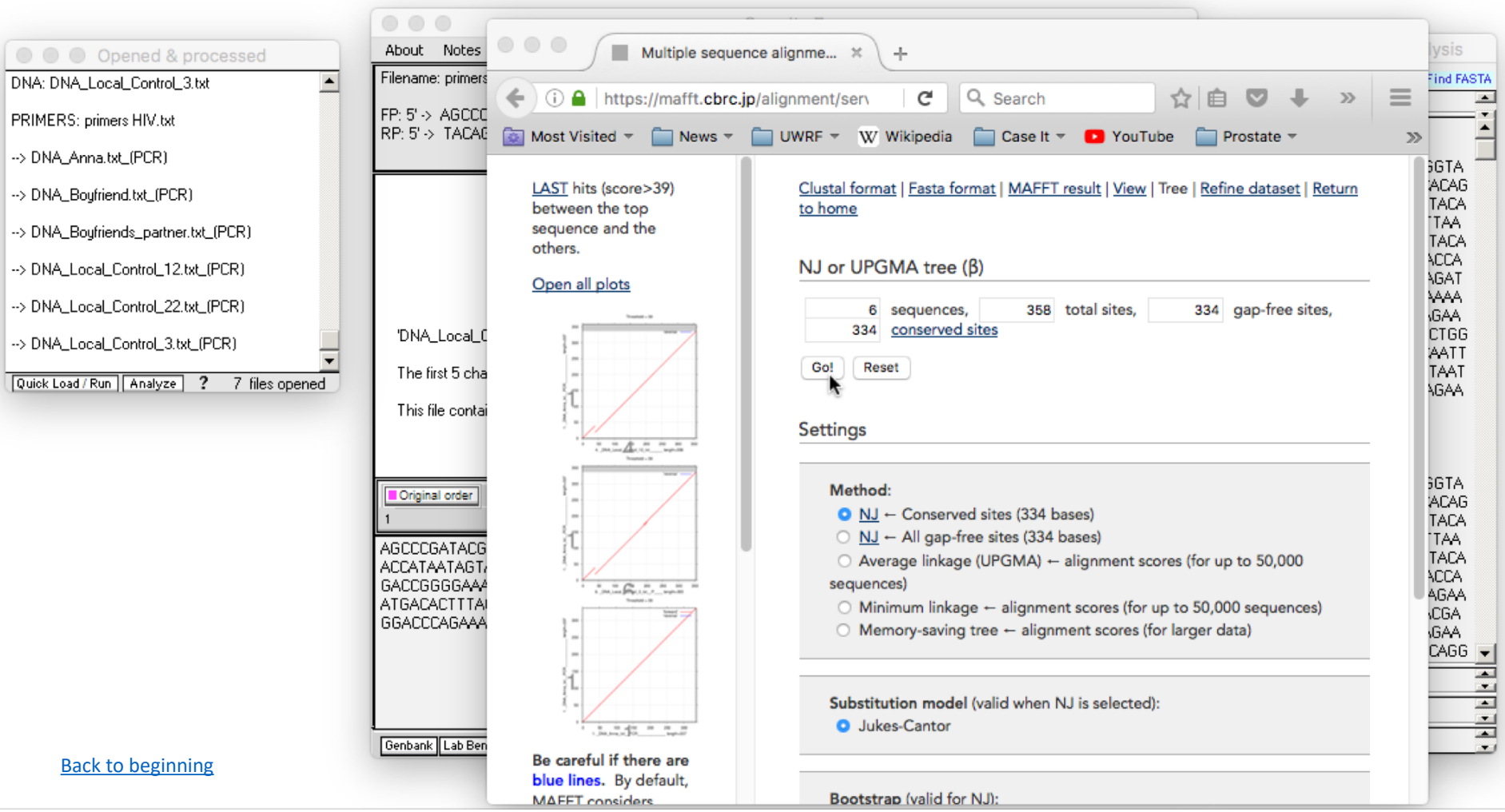
Default settings will be used in this example, so click the **Go** button…



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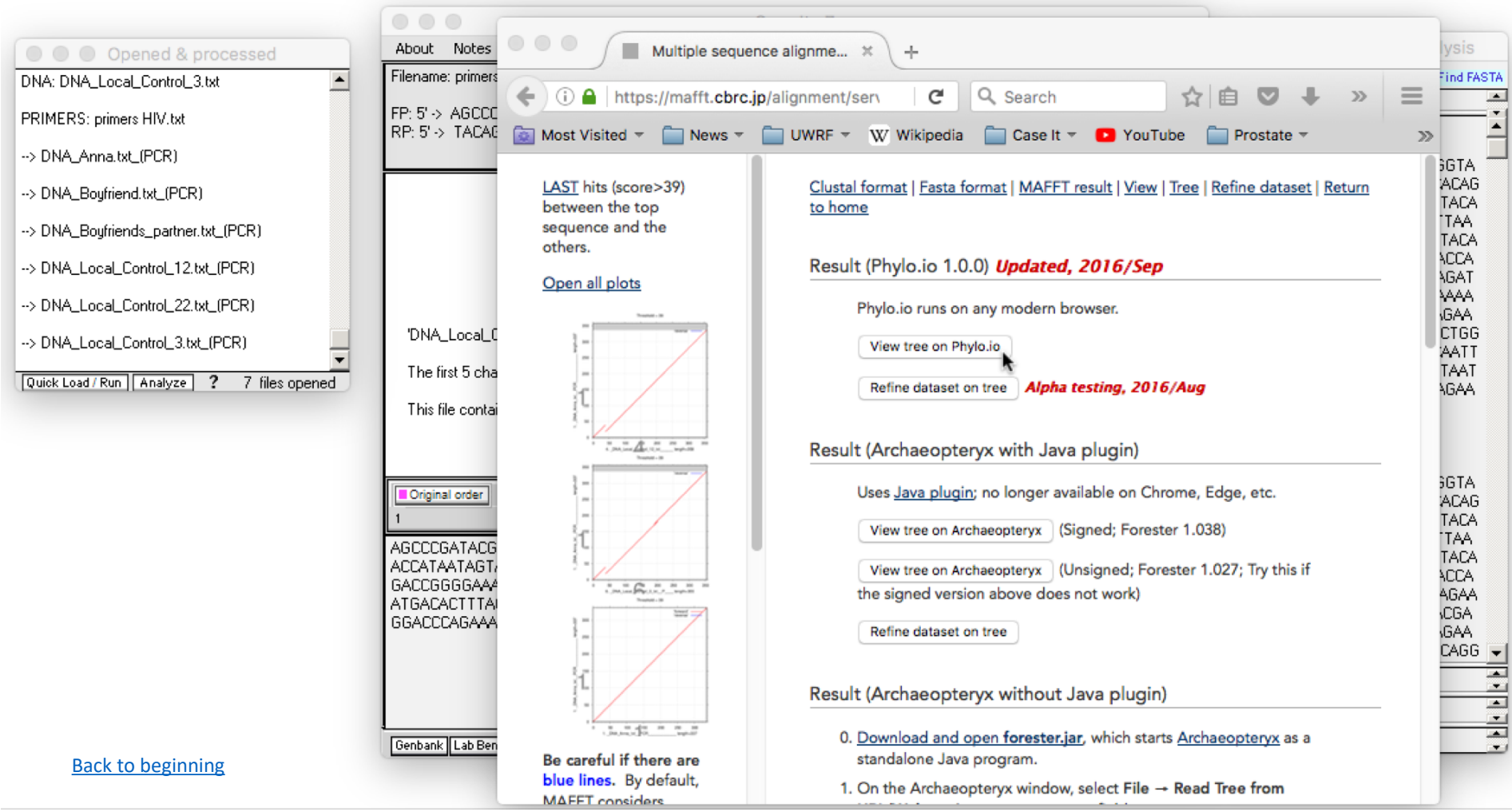
…click **View tree on Phylo.io**…



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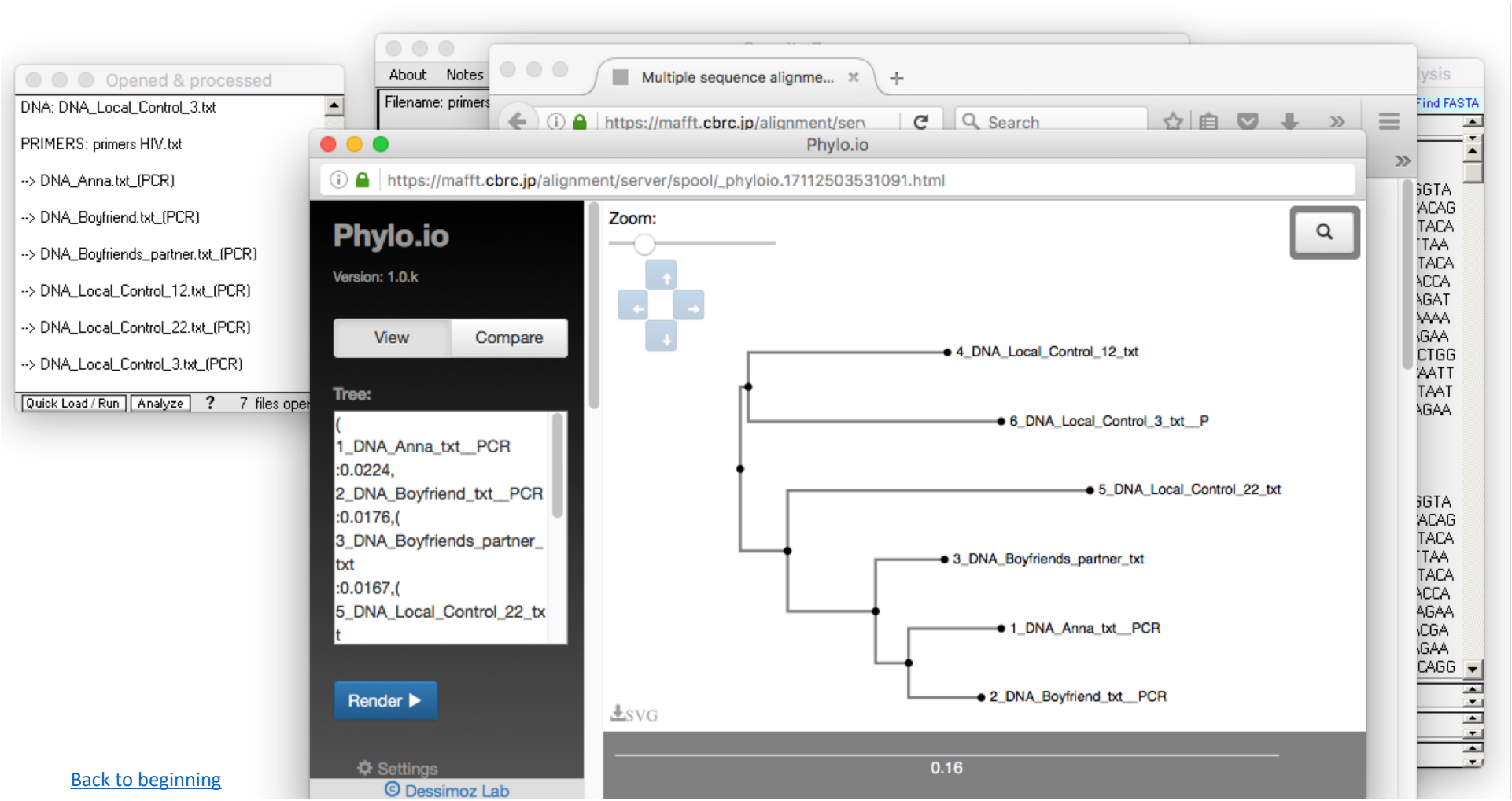
…and the tree will appear. In this example, the directional arrow buttons were used to change the original scale of the tree.



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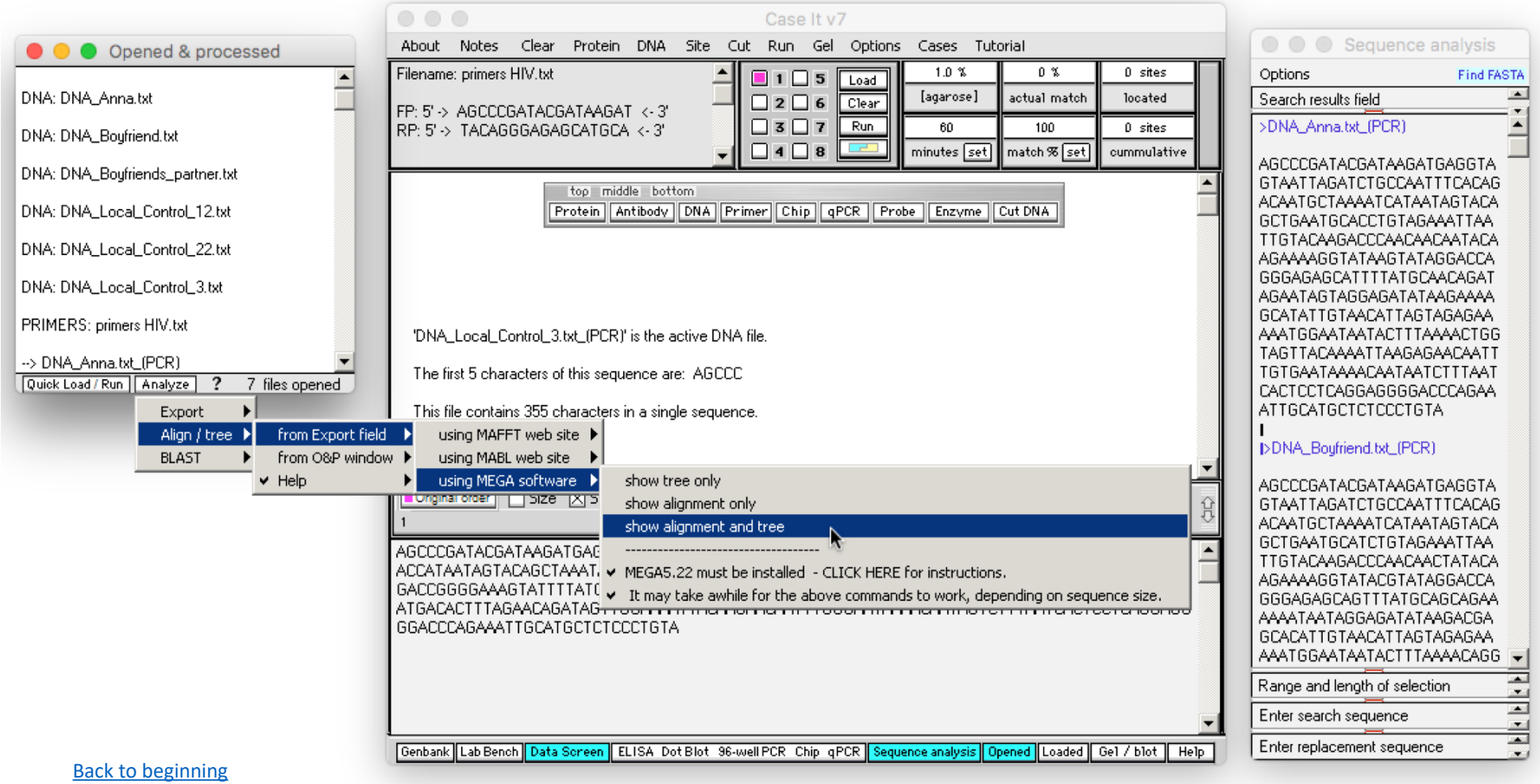
The third option for multiple alignment and tree-building is to have Case It open and control MEGA5 software. If default values for this software are used, then Case It will open MEGA5 and build a tree with one click (assuming that the MEGA5 executable is in the MEGA folder of Case It – see "Installation and Overview of Case It" on how to obtain MEGA5). Click the **Analyze** button and select the menu commands below.



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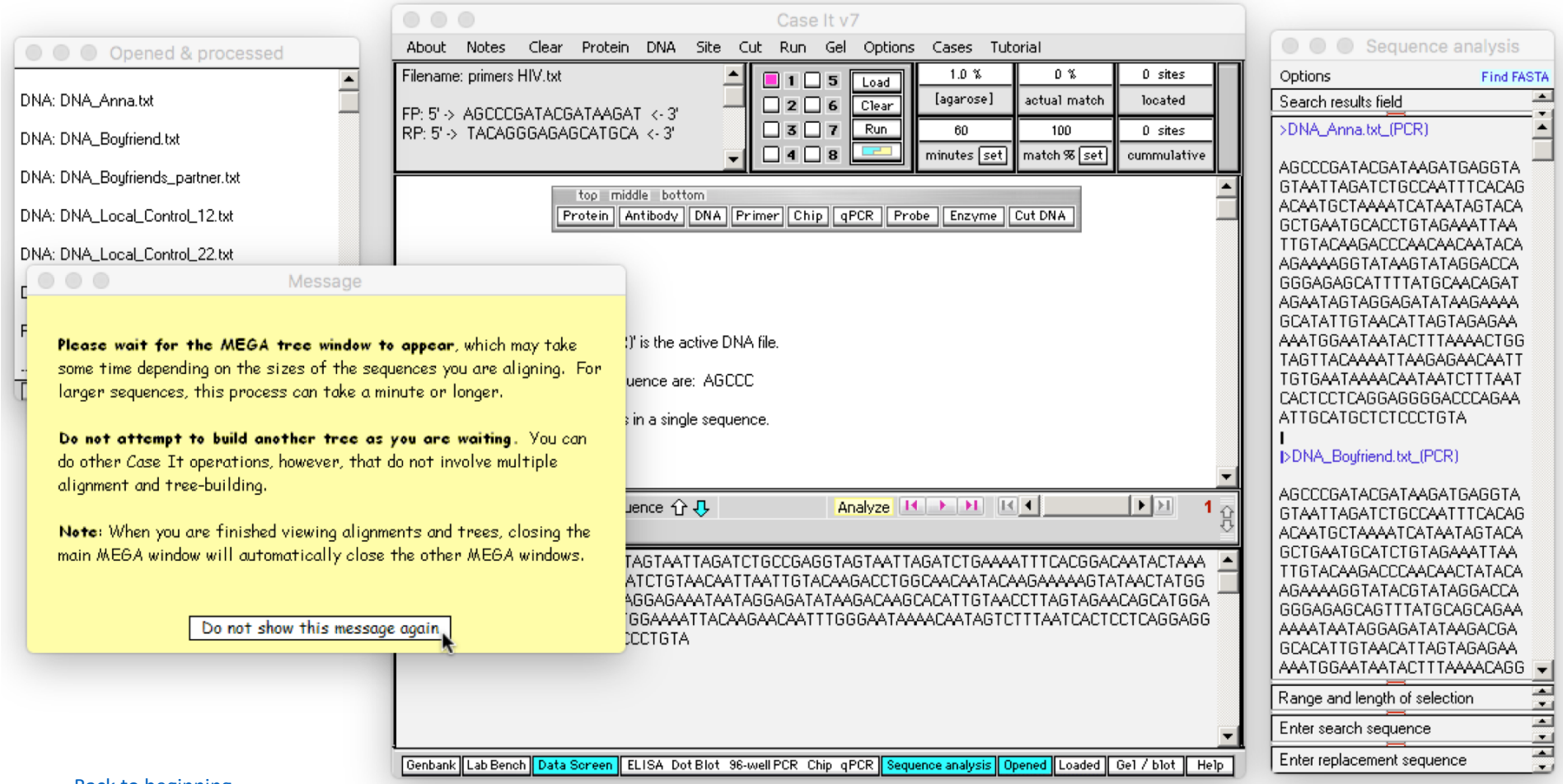
The first time that this command is used, a yellow alert box will appear, indicating that it will take some time for MEGA to appear. This depends on the speed of your computer, and the number and size of sequences being aligned. So the key is to be patient. Note that other Case It operations can be conducted while you are waiting, but that you should not attempt to build another tree until the first one appears.



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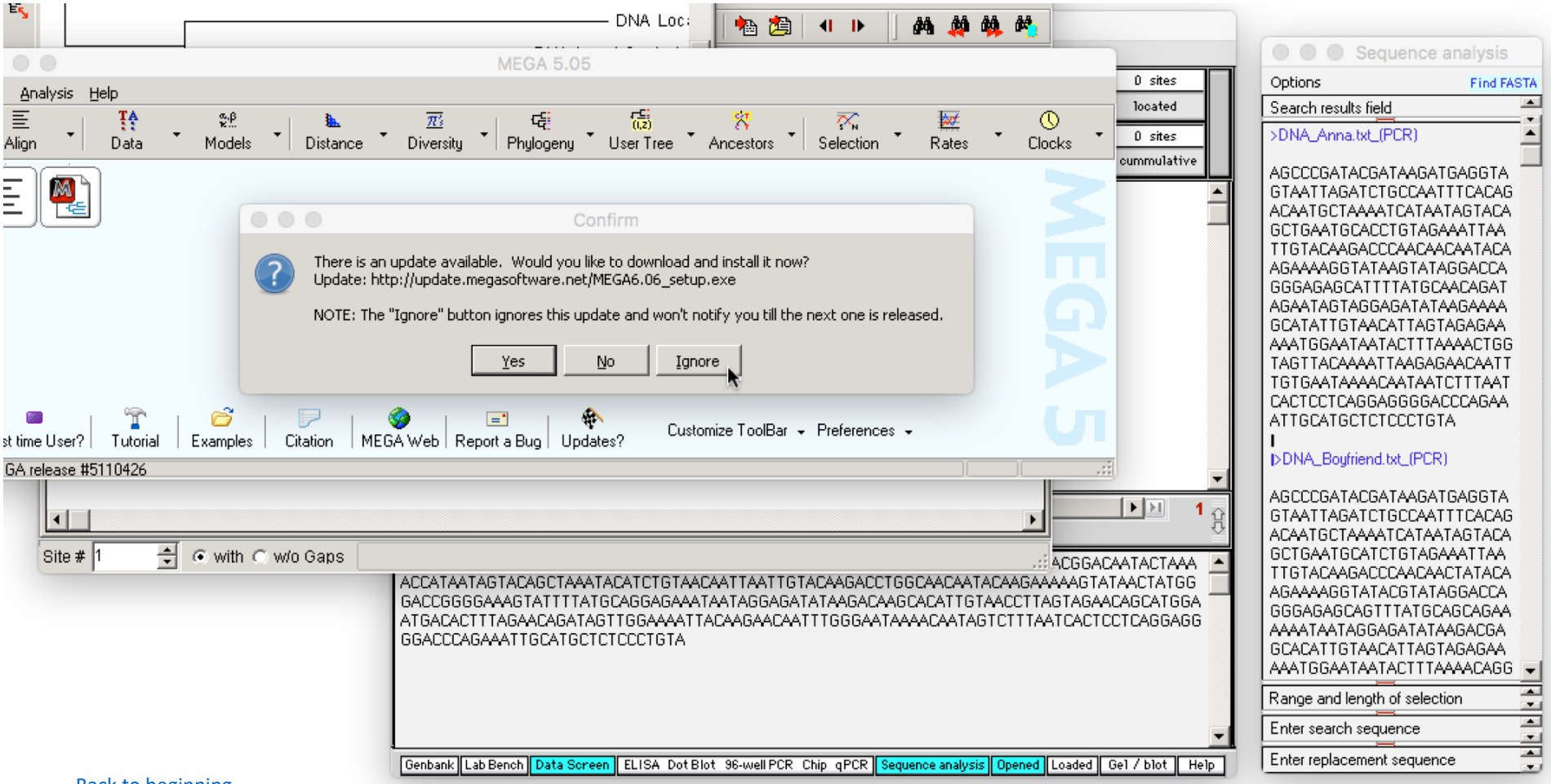
Several windows will open, including the main MEGA5 window (the one with the light blue background). On the Confirm window, click **Ignore**, as you don't want updates to MEGA5 since they won't work with Case It. After clicking Ignore, **minimize the light blue window**. (Minimize it, don't close it, as closing this window closes all windows of MEGA5).



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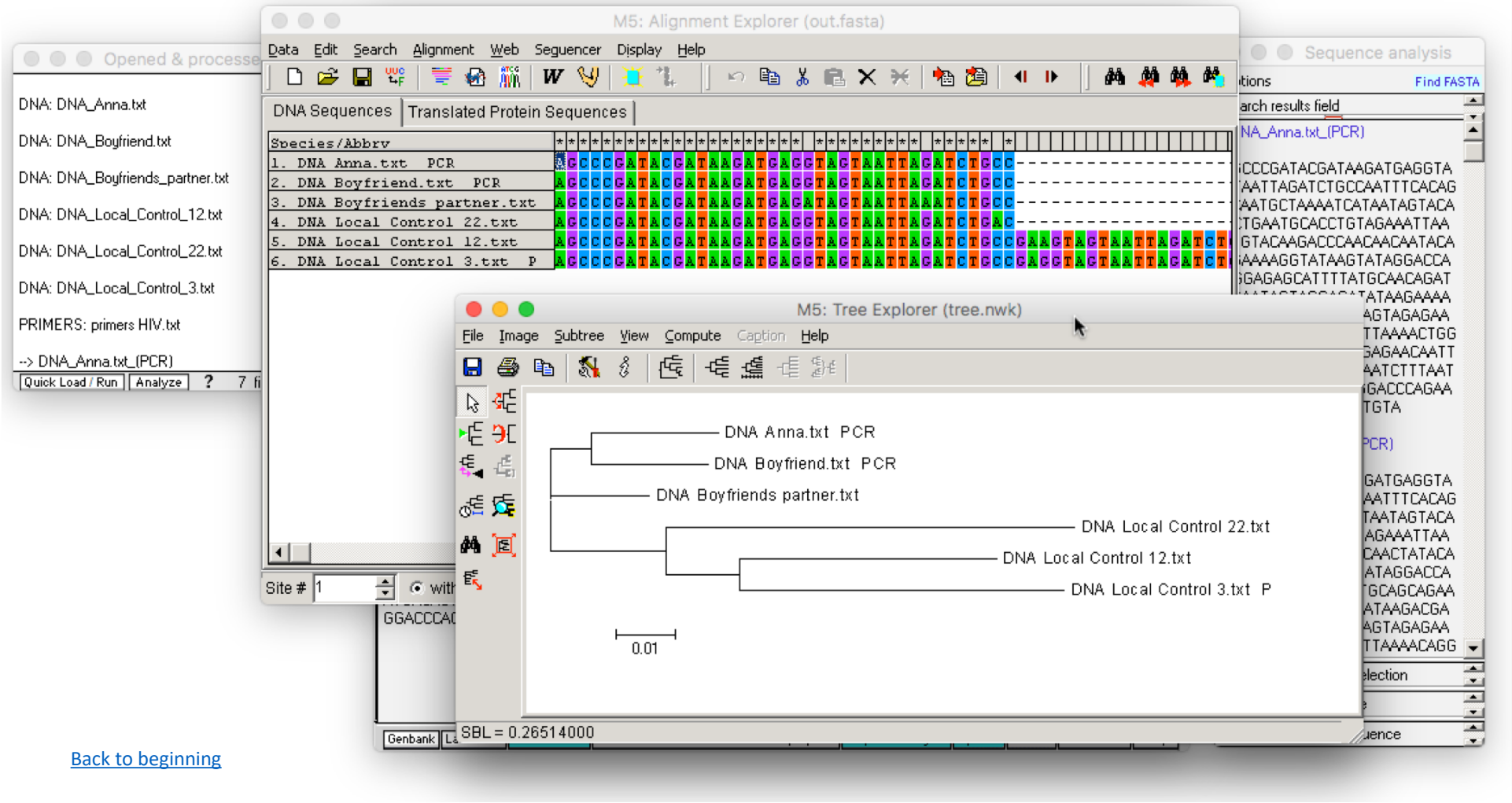
The multiple alignment and tree now appear. The advantage of using MEGA5 over the bioinformatics sites has do do with the many manipulations of the alignment and tree that are possible using MEGA5.



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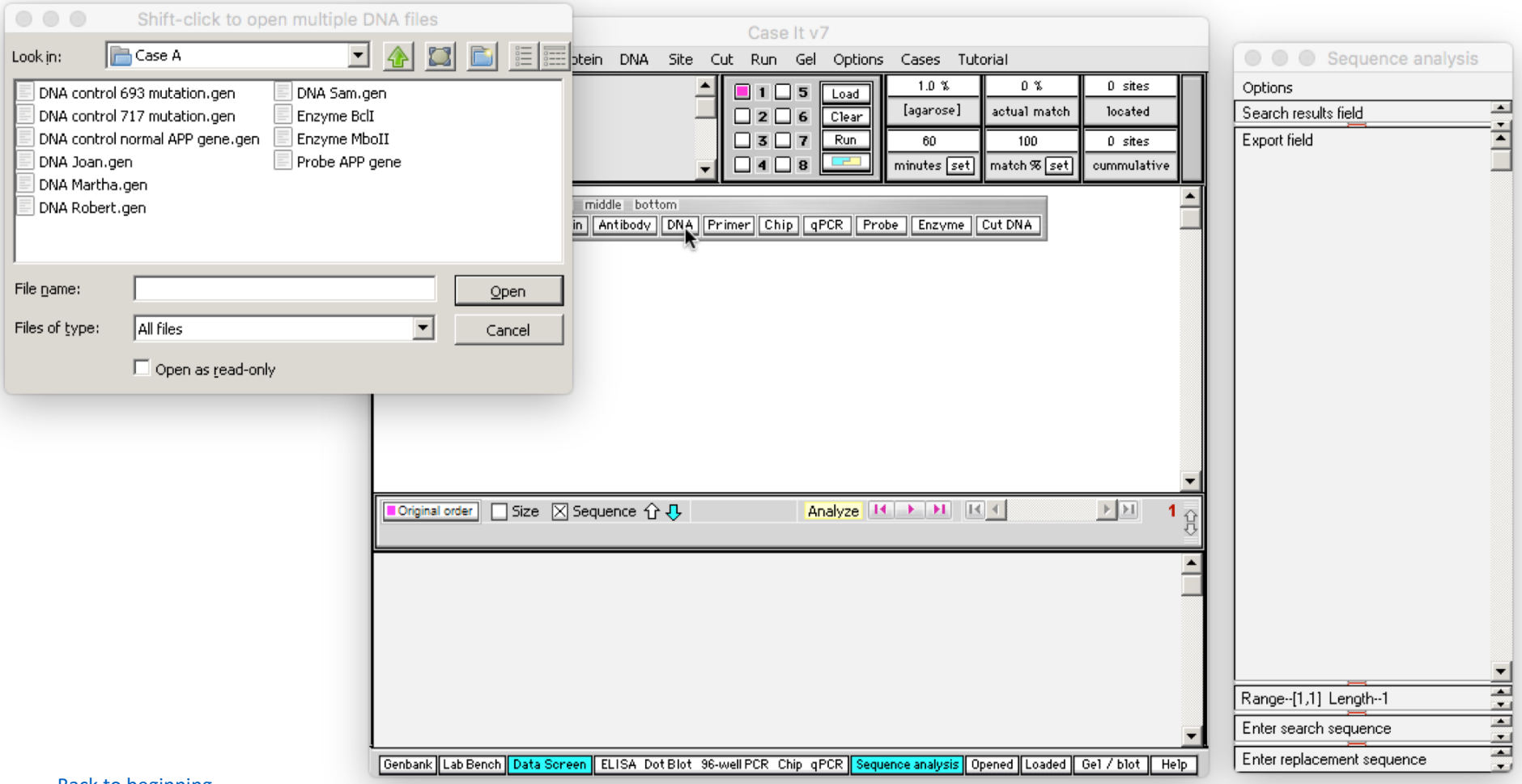
Case It can also be used as a front end for BLASTING DNA and protein sequences. We'll use one scenario from the Alzheimer's case as an example. Click the **DNA** button on the silver button bar, and navigate to **Cases -> Genetic disease cases -> Alzheimer's -> Case A**.



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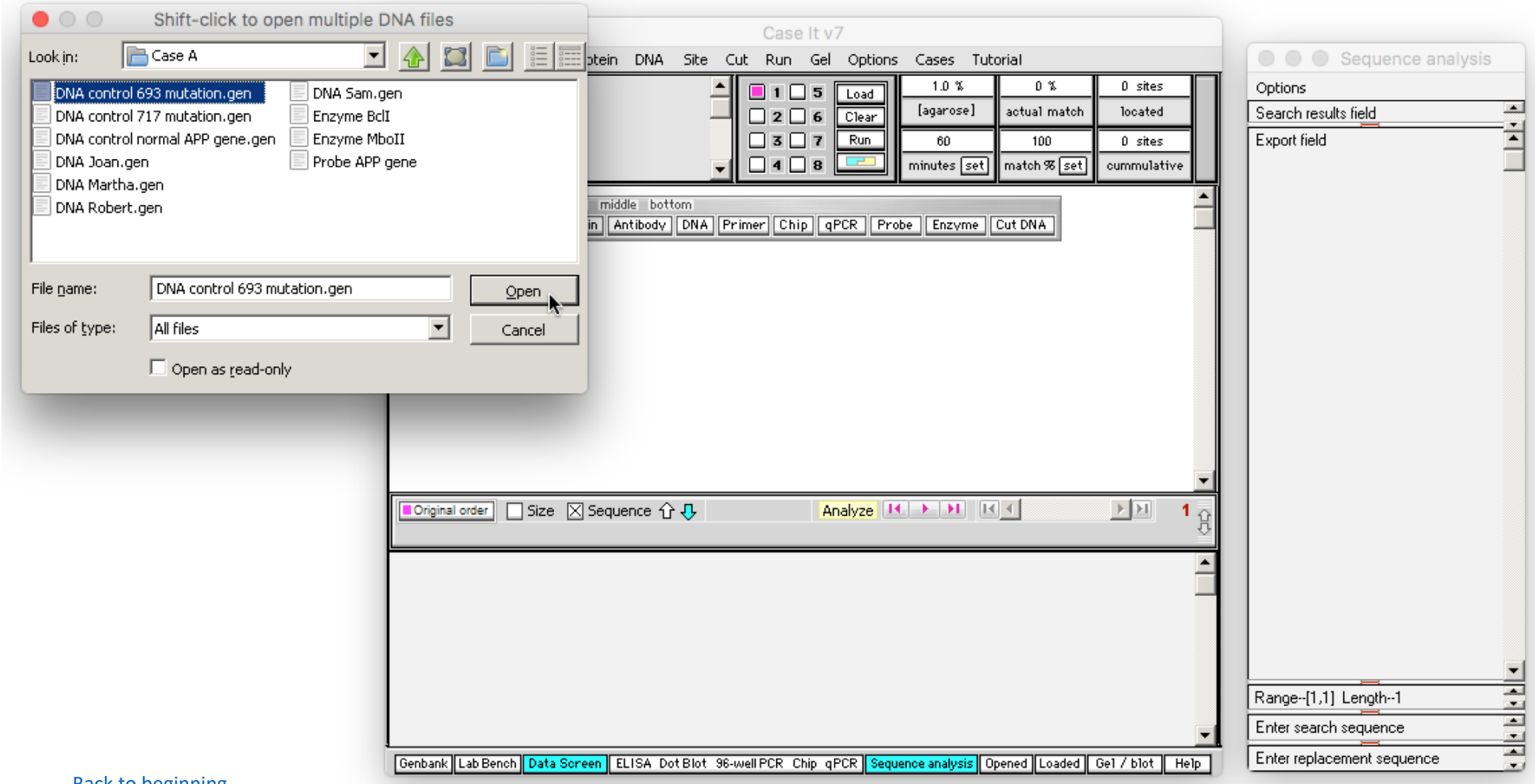
Any of the DNA sequences can be selected, so for this example we'll select the first one. Double-click on the first file name, or click once and click the **Open** button…



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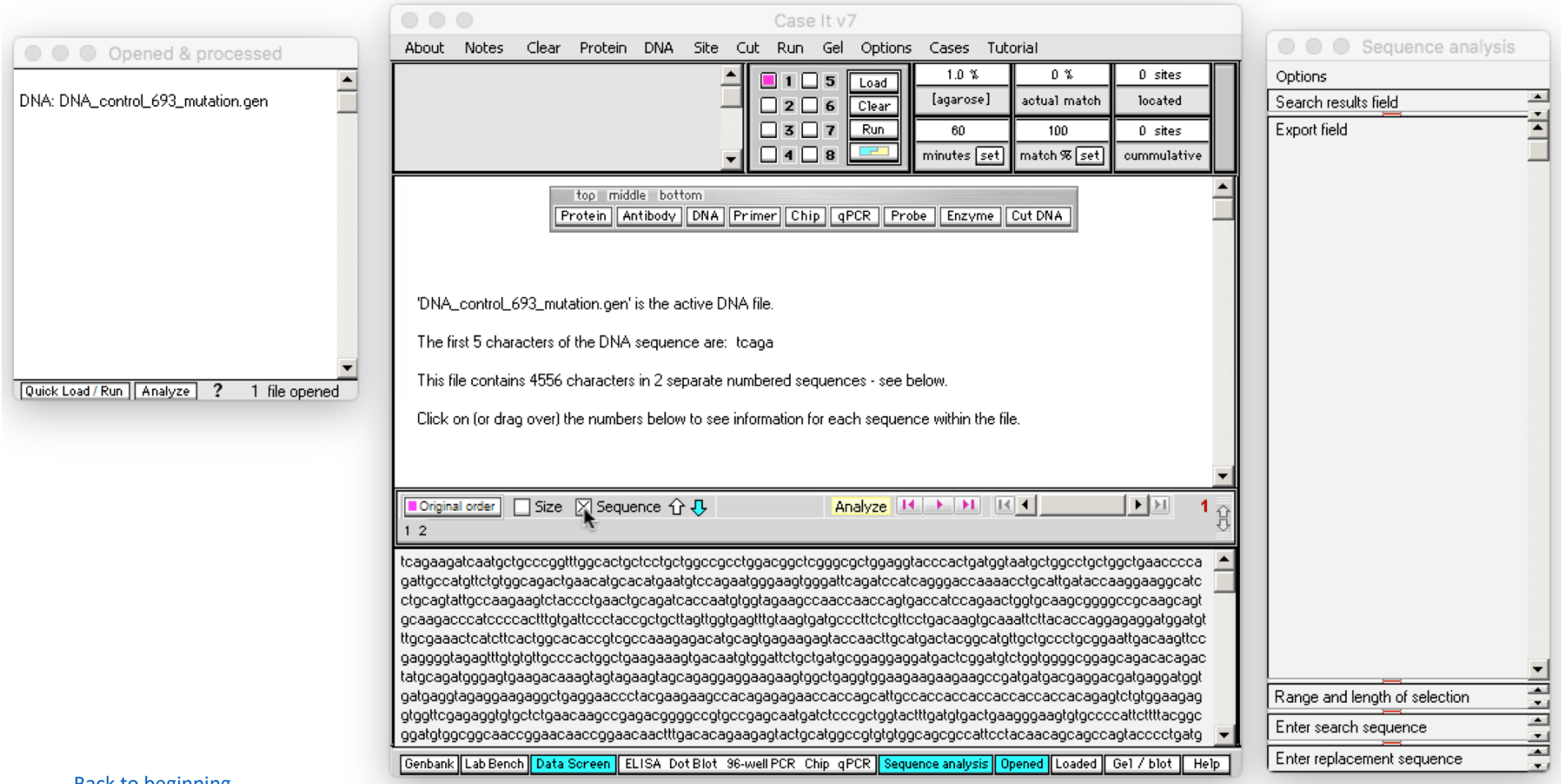
The name of the file appears in the Opened and processed window at the left. To see the sequence associated with this file name, click on the **Sequence** checkbox on the gray divider bar. By default, the blue arrow to the right of the checkbox points down, indicating that the sequence in the lower field will be shown.



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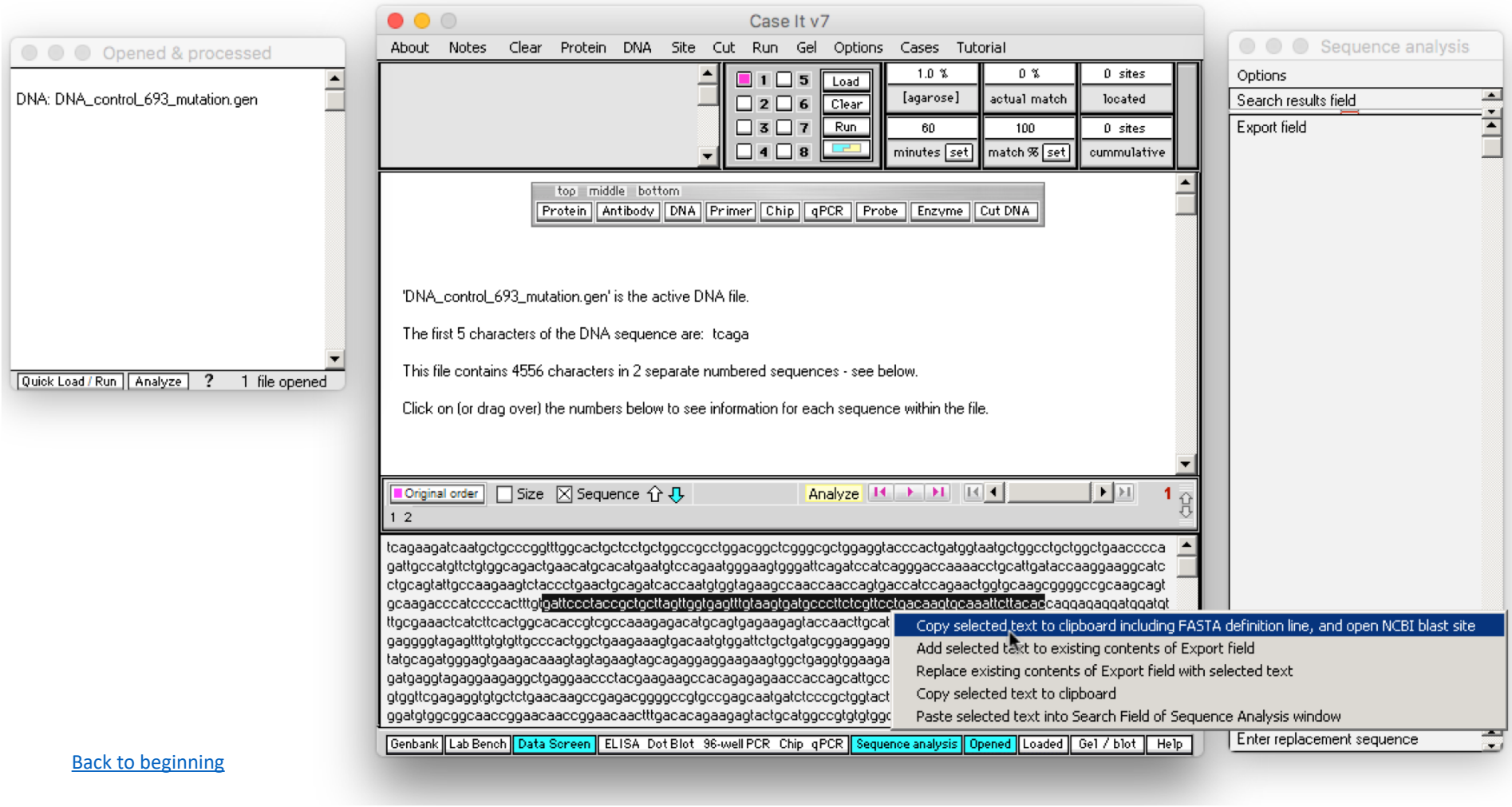
Highlight any part of the sequence and right-click on it. Select the first menu option in the pop-up menu to automatically open your default web browser the the NCBI blast site.



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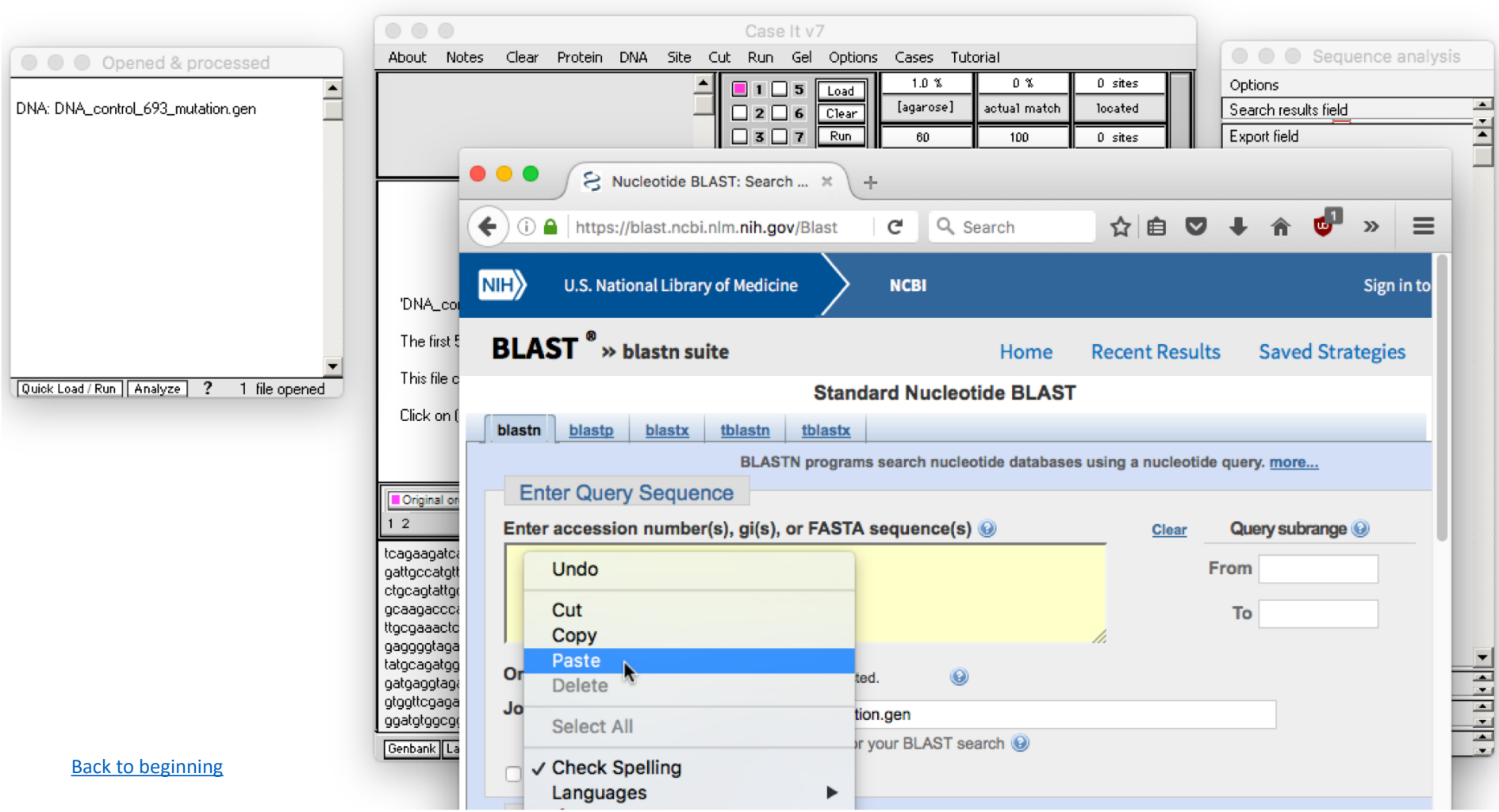
Right-click in the Query Sequence field of the NCBI site, and paste the contents of the clipboard into the field. It may be necessary to click and paste twice for this to work.



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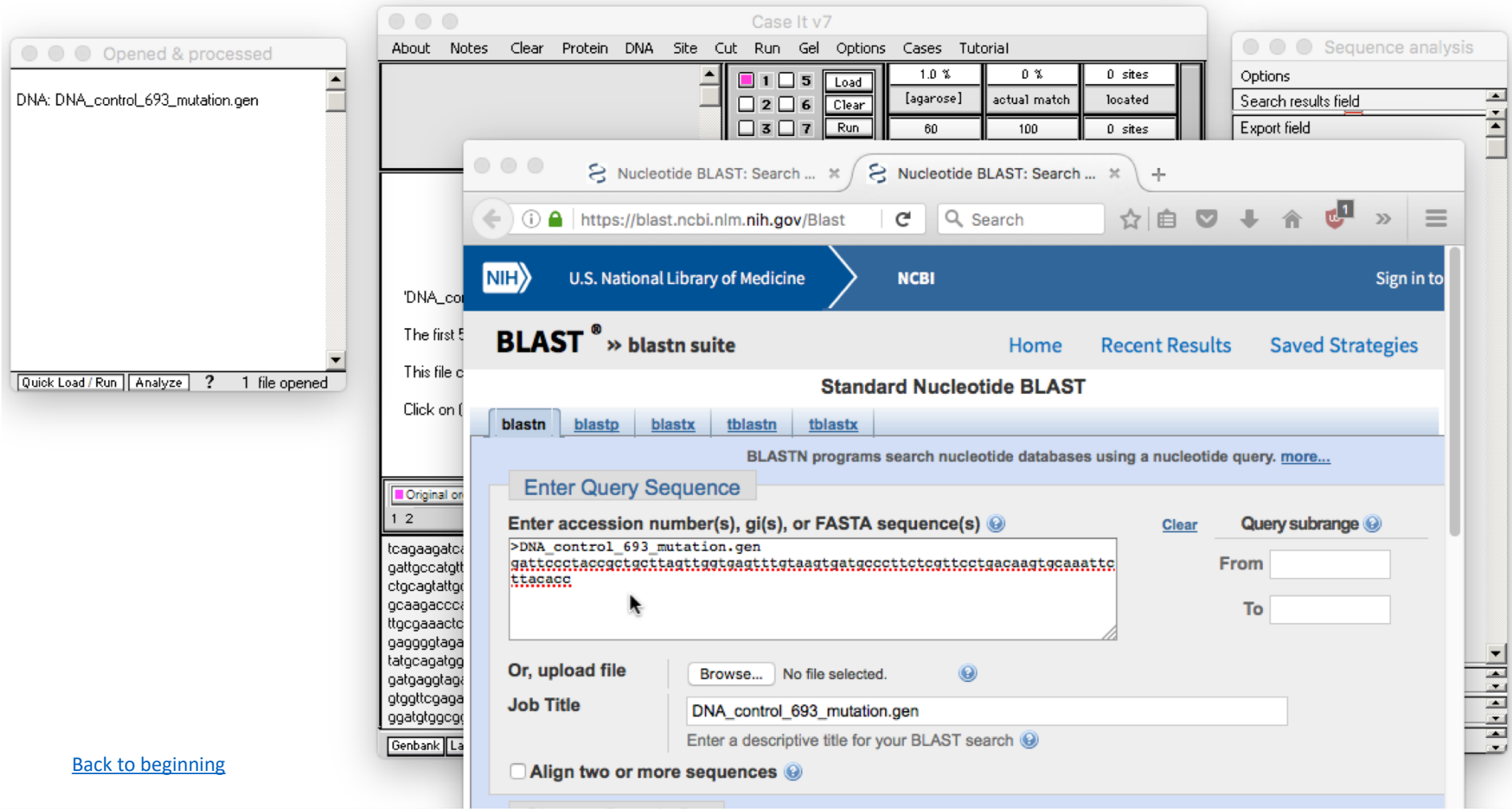
The DNA sequence copied from Case It is not in the Query Sequence field of the NCBI site.



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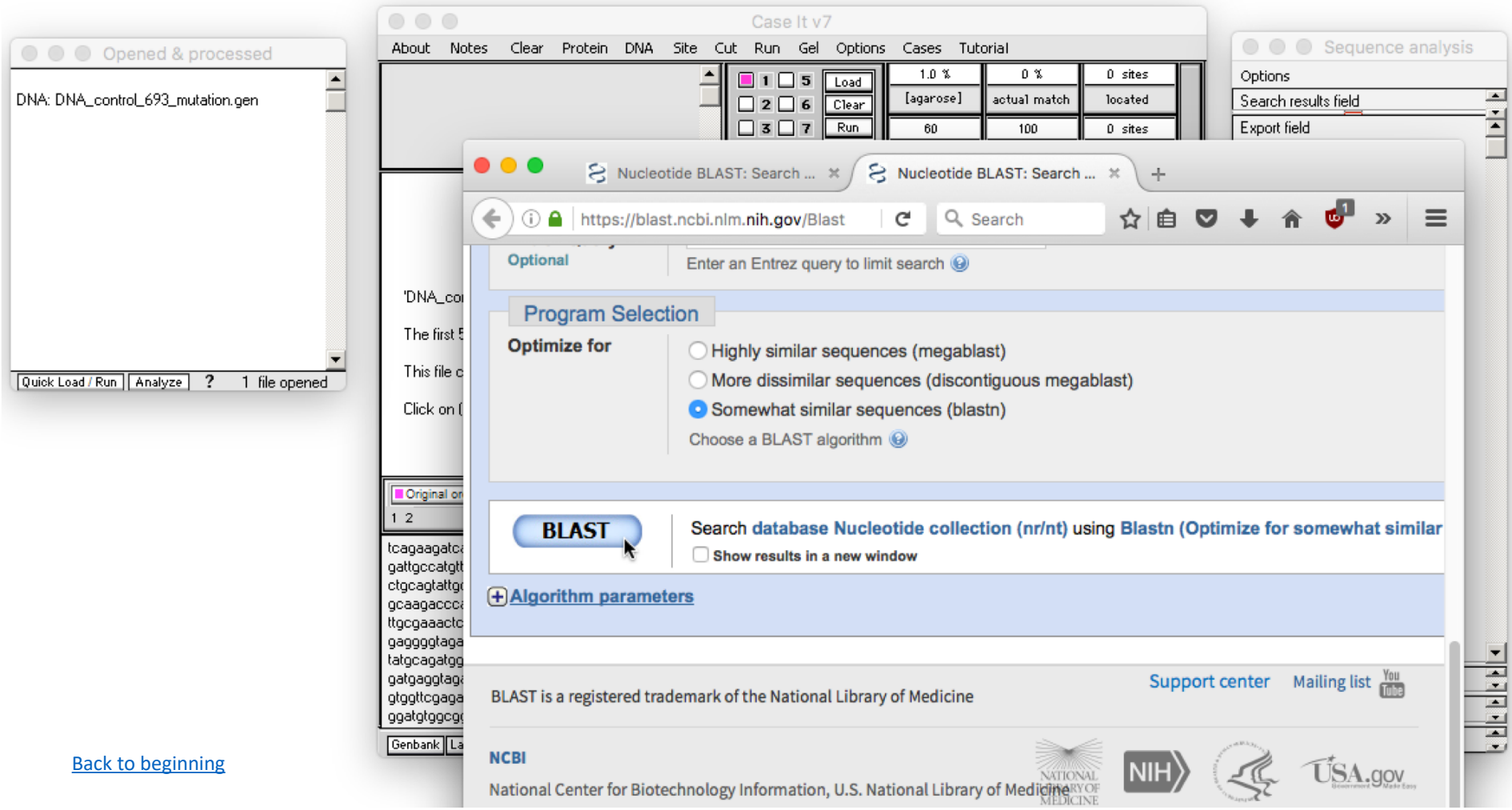
Scroll down on the NCBI page and click the **BLAST** button.



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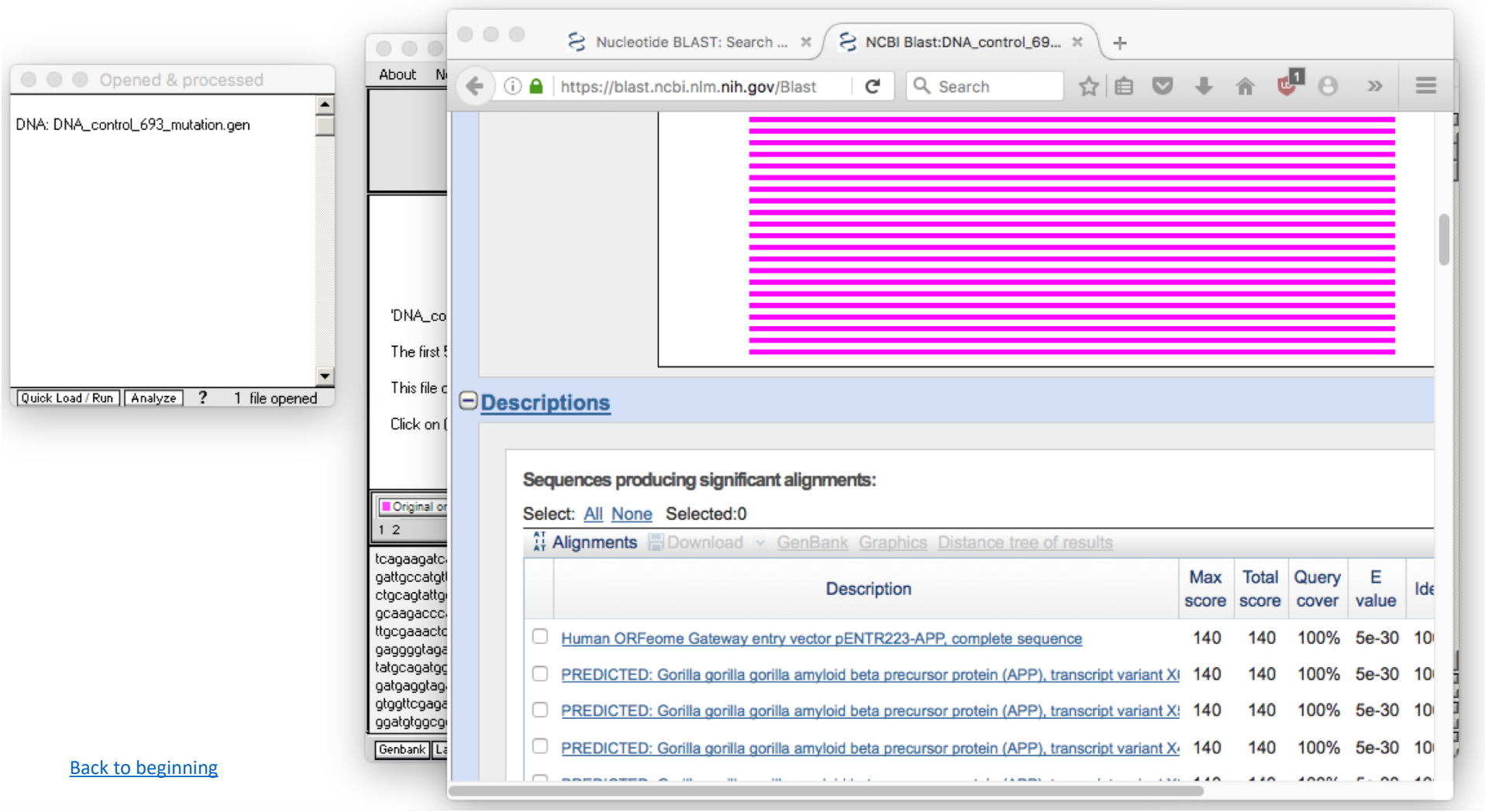
After a few moments the BLAST results will appear.



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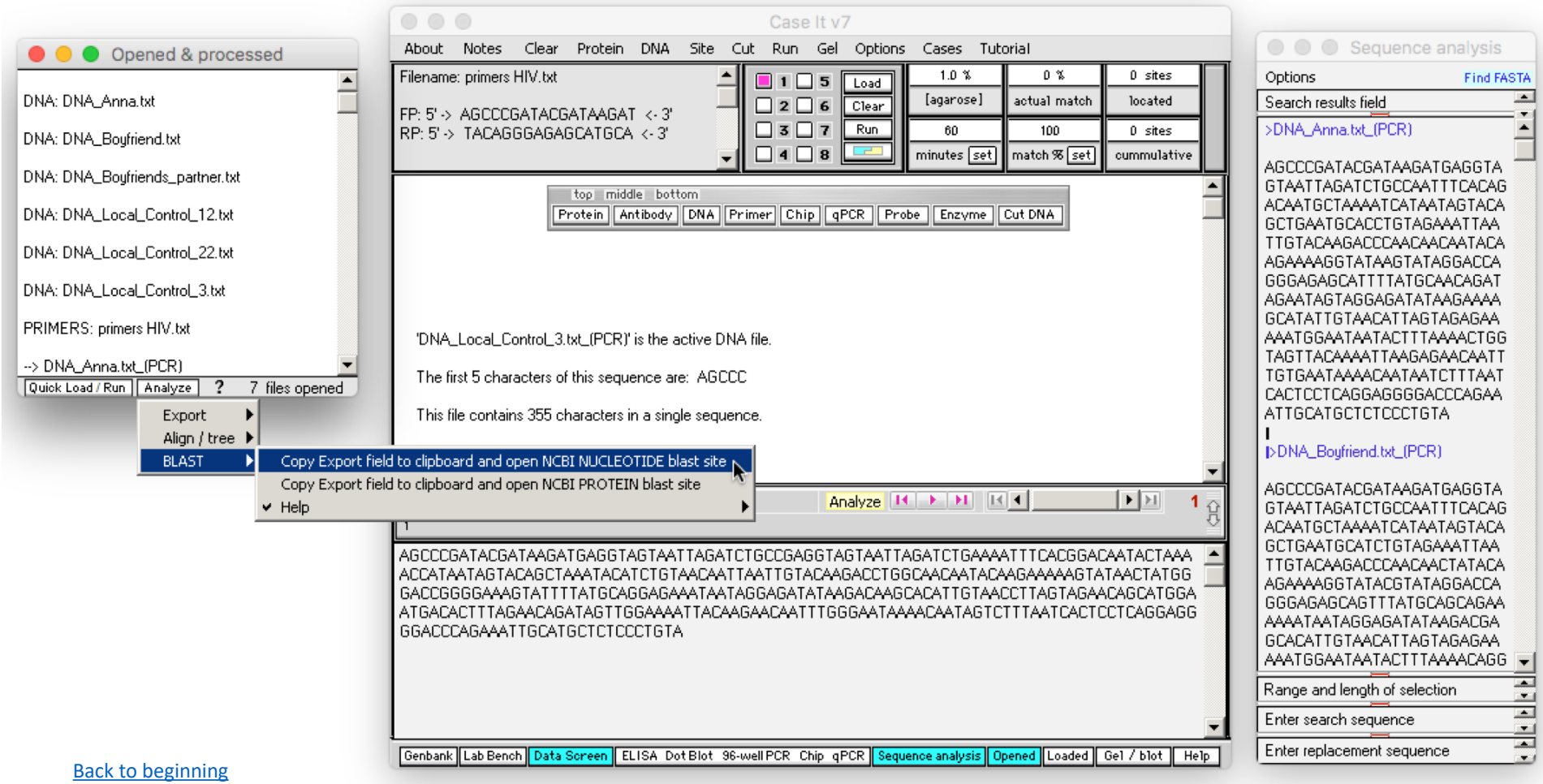
BLAST can also be used to analyze data in the Export field, using several methods. To demonstrate the first method, we'll use the HIV example described earlier, assuming that sequences have already been added to the Export field (see pp. 2-8 of this tutorial).

Click the **Analyze** button at the bottom of the **Opened & processed** window, and make the menu selection shown below.



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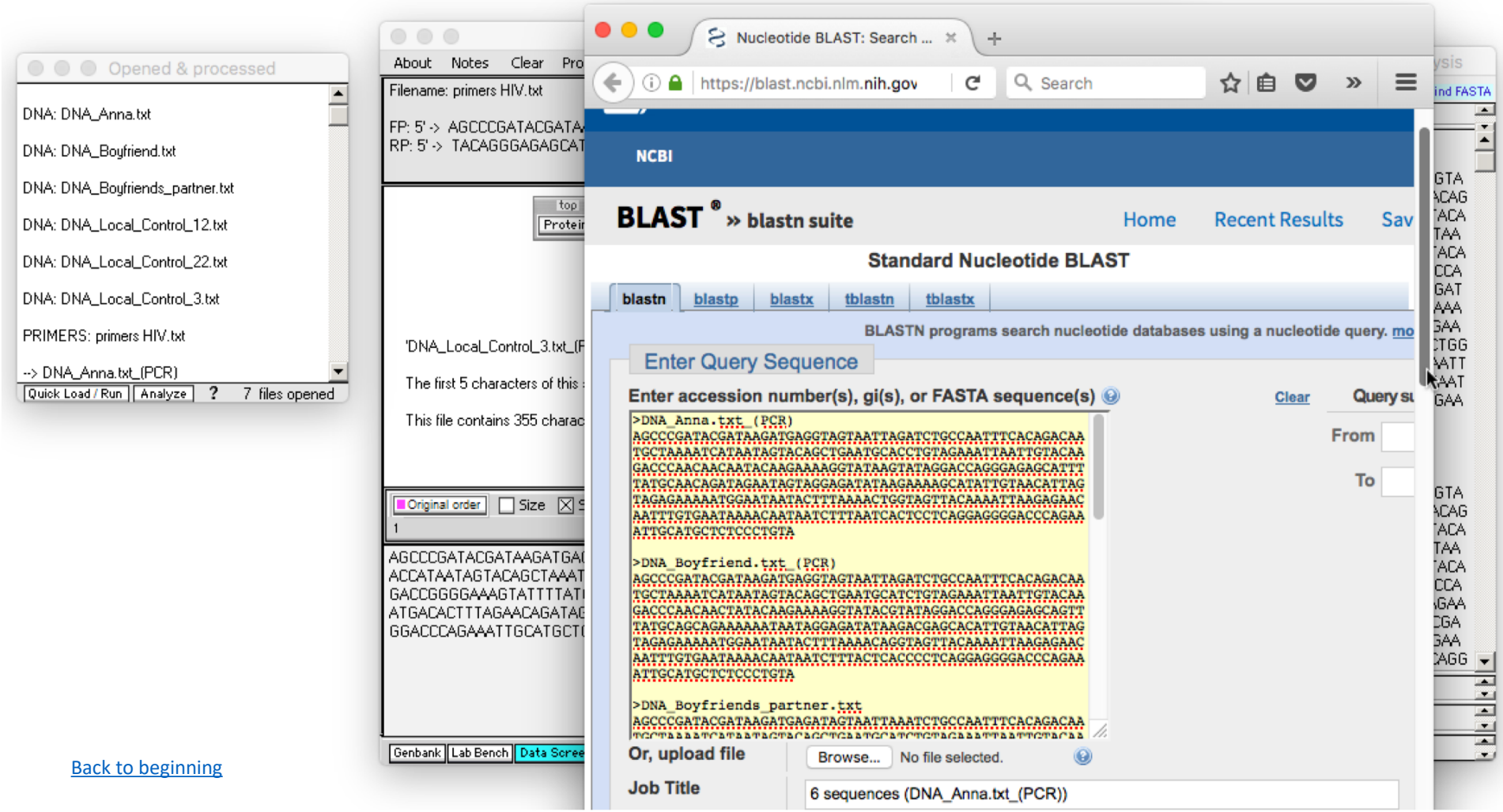
The NCBI web site will automatically open, and you can paste the contents of the clipboard into the Query Sequence field by right-clicking on the field and selecting Paste (you may have to do this twice). Then scroll further down the web page and click the BLAST button (not shown here).



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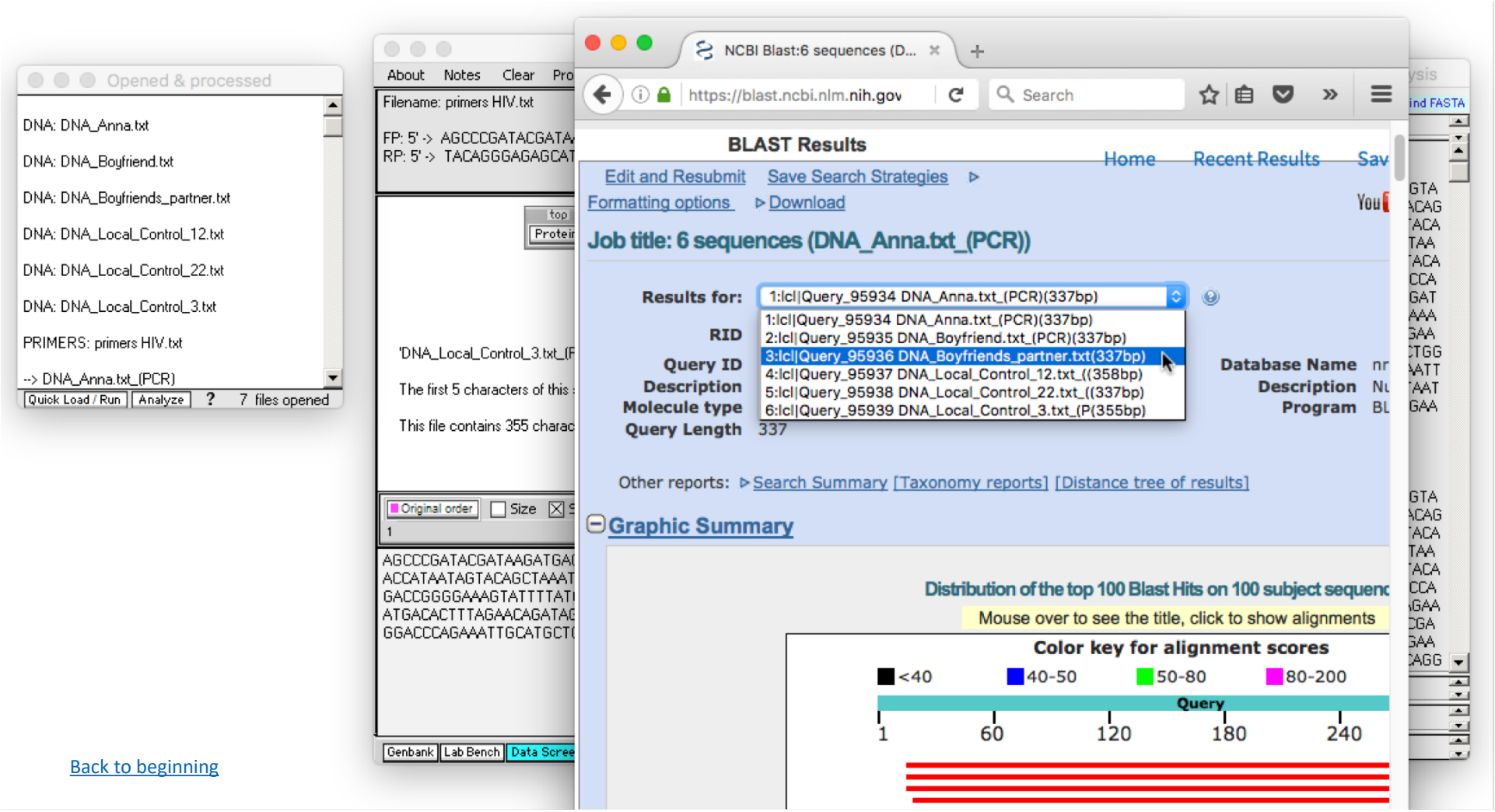
Since multiple sequences were BLASTed, you can select the results you wish to view from the drop-down menu on the BLAST Results page.



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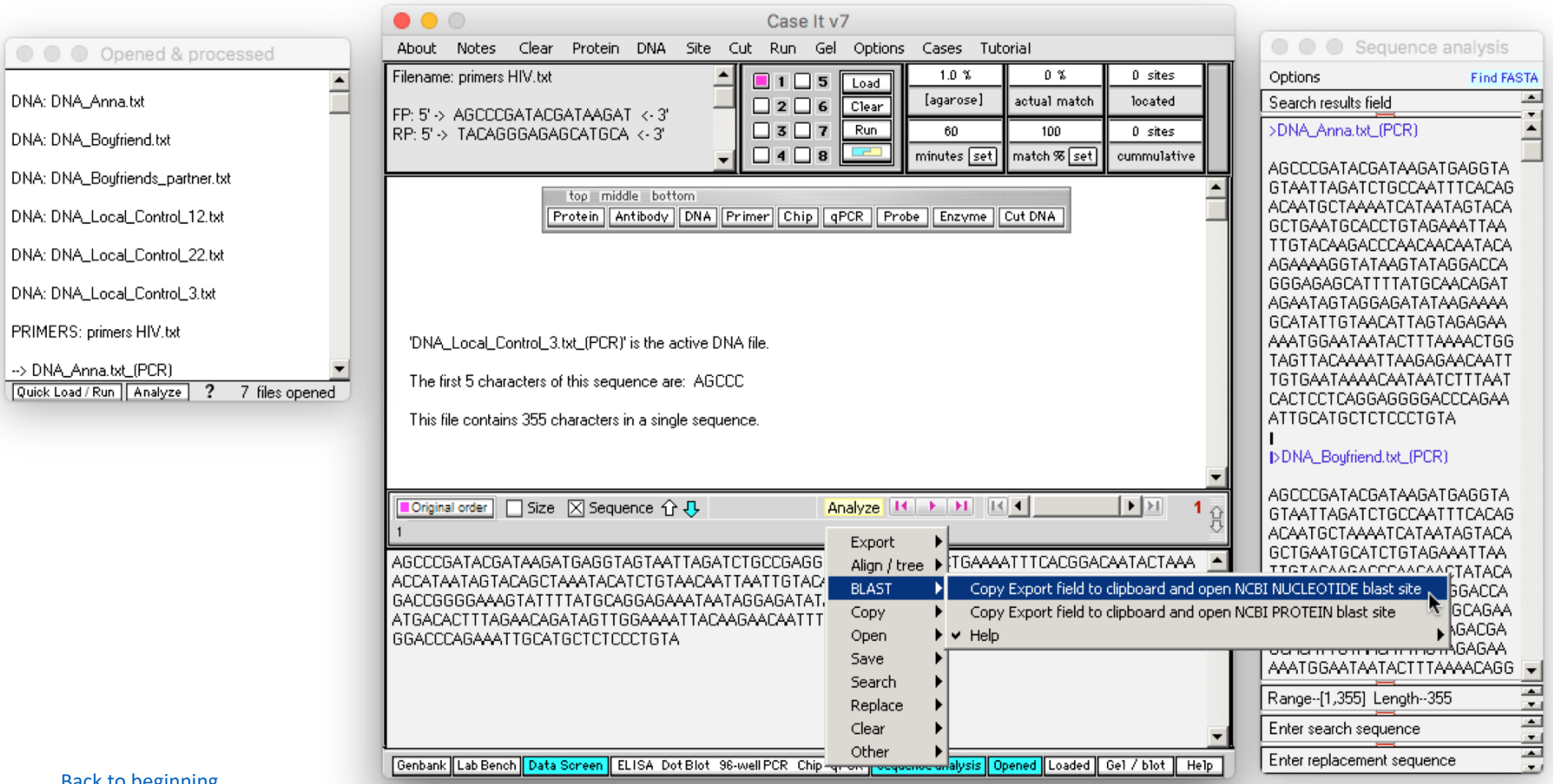
Another way to accomplish the same thing is to use the yellow Analyze button on the main screen. Note that the two Analyze buttons have some commands in common, but some unique commands as well. In this particular case, BLAST results would be identical to those shown on the preceding page of this tutorial, so we won't show them again.



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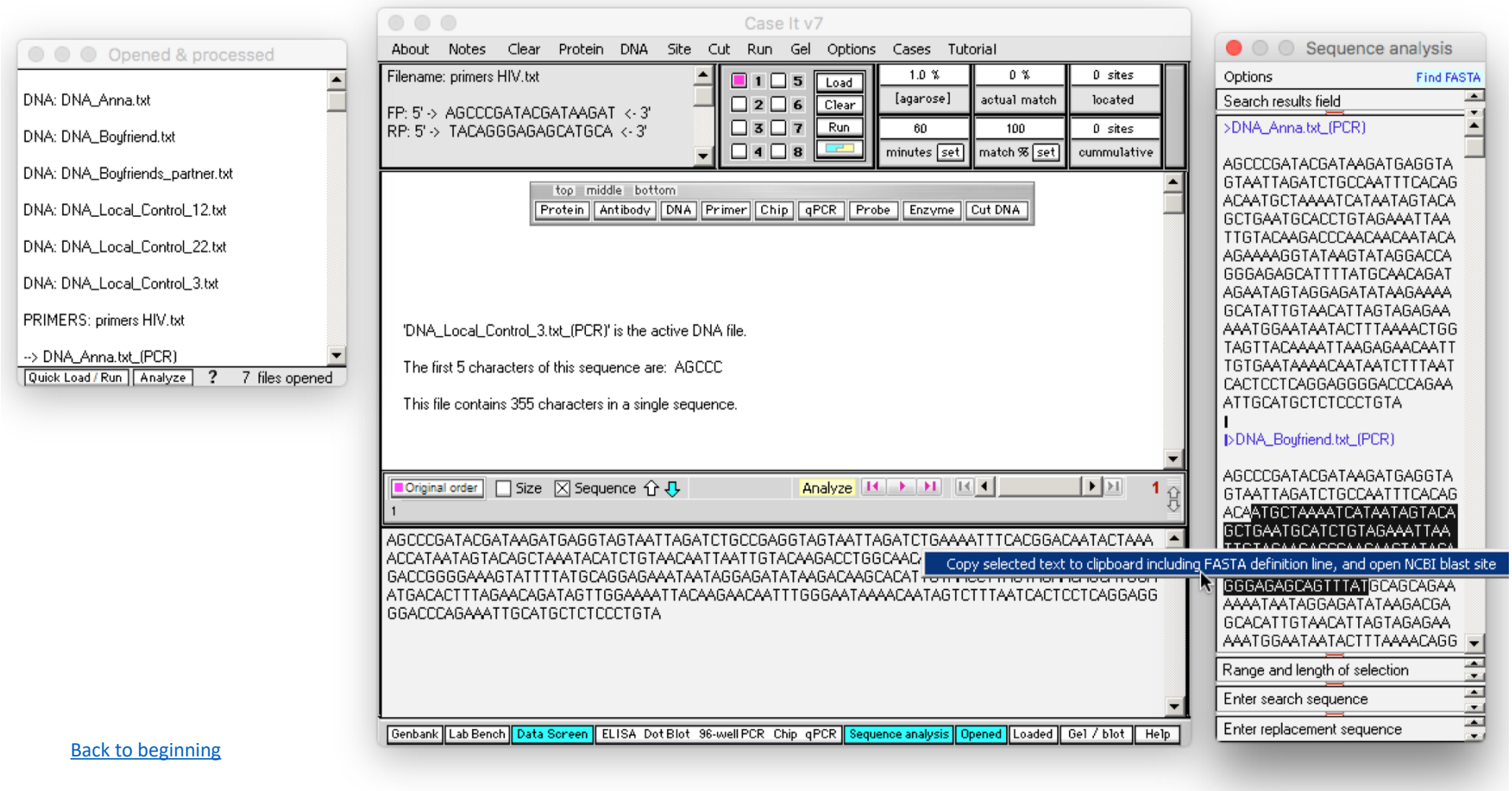
You can also highlight and BLAST highlighted contents of the Export field, by right-clicking on the highlighted sequence. We won't show the BLAST results for this particular example, but it would be similar to those shown previously.



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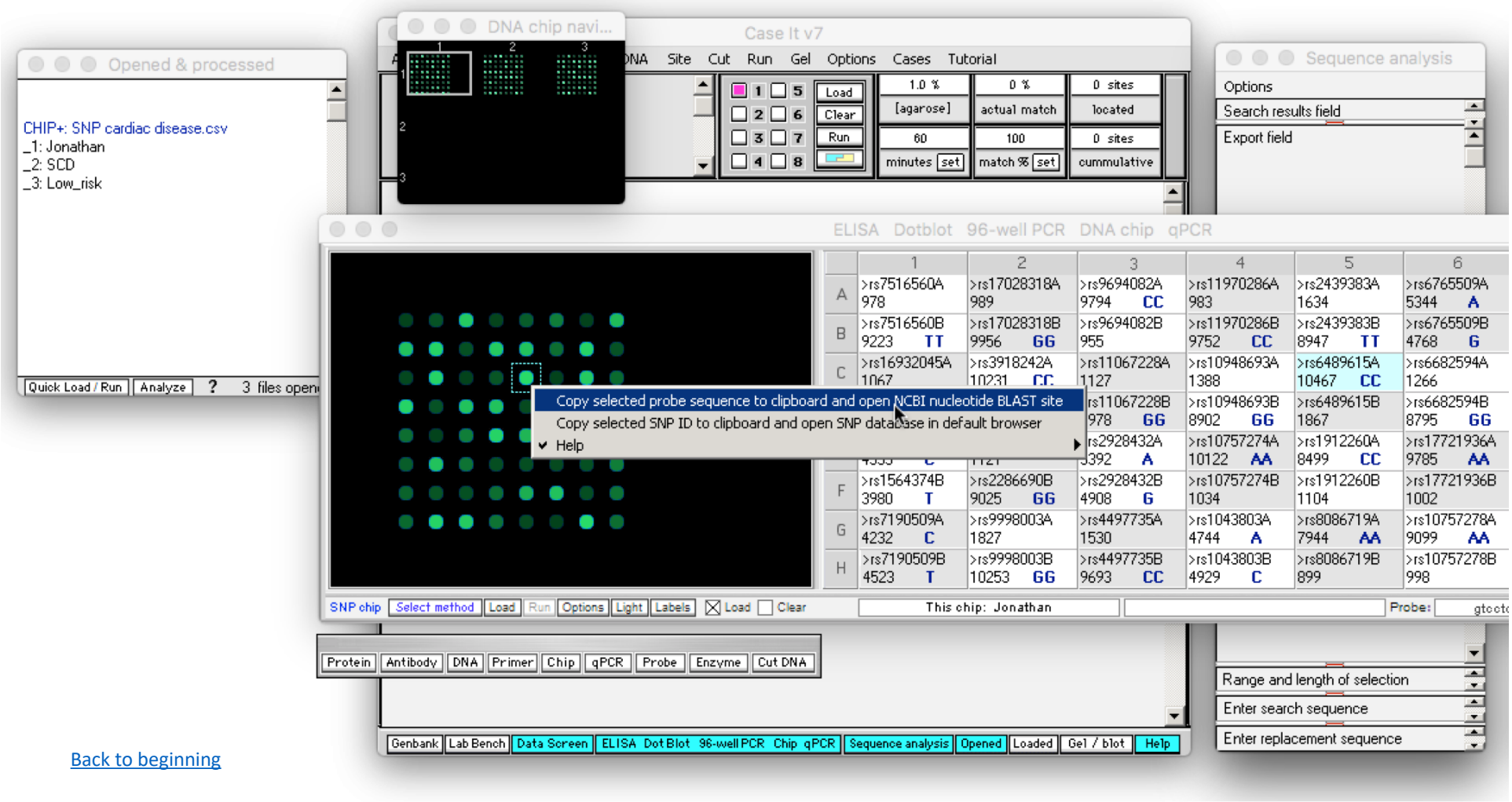
BLASTing can also be done on features of a microarray, by right-clicking on a selected feature. The procedure for setting up a SNP or expression microarray can be found in a separate tutorial. This concludes the tutorial on BLASTing sequences via Case It.



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