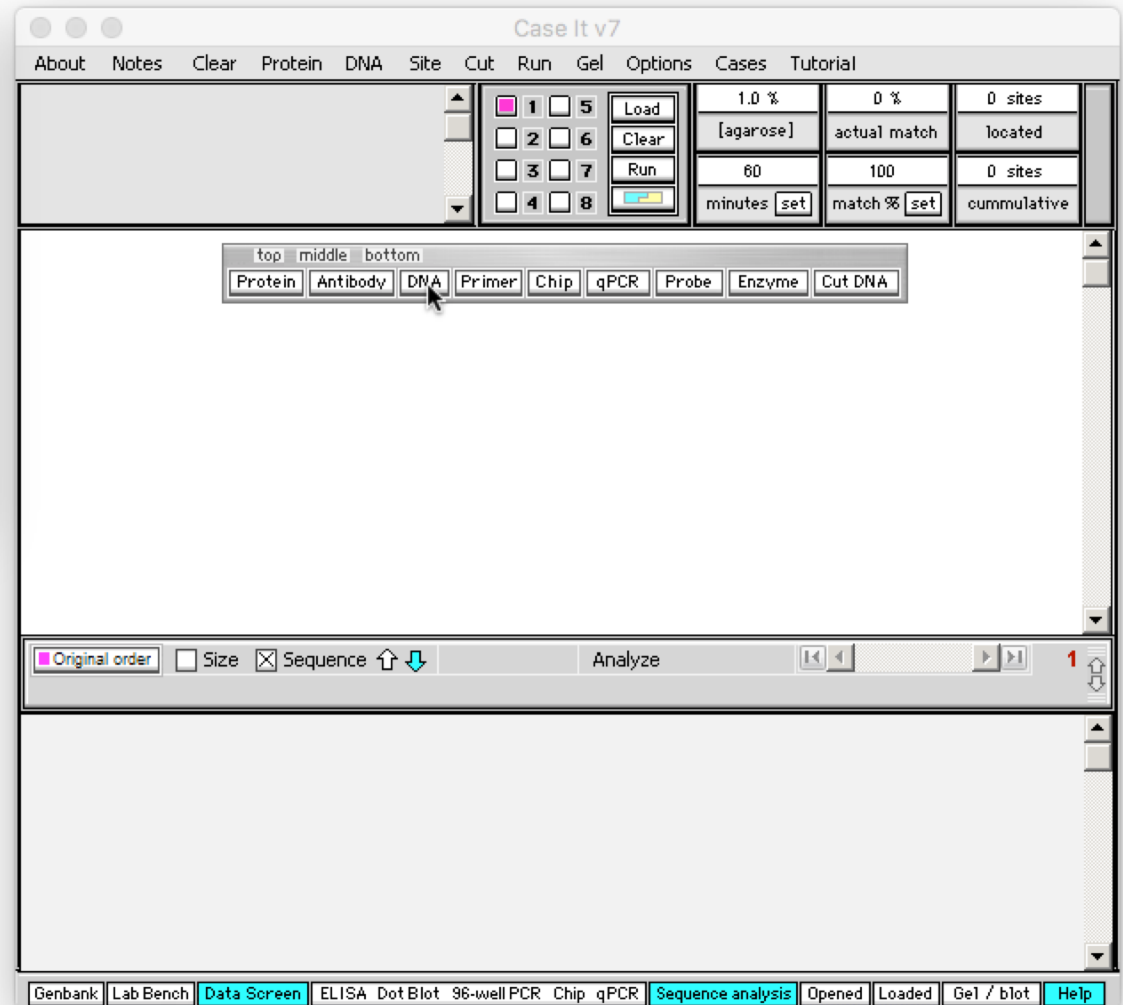
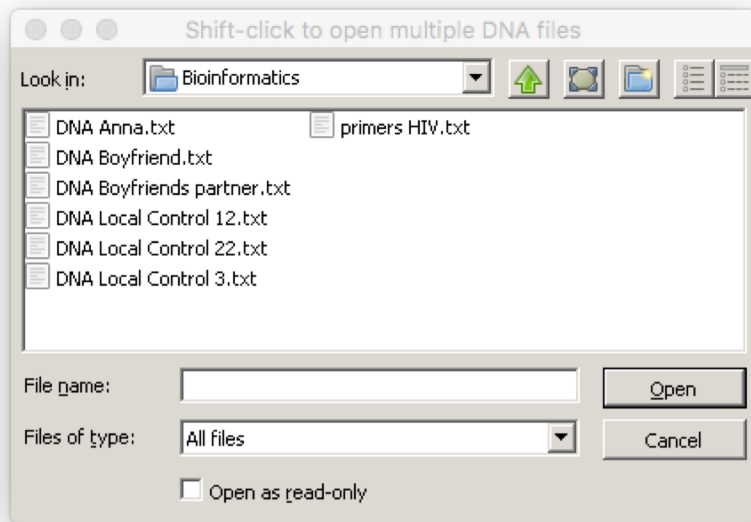


Tutorial for using Case It for bioinformatics analyses

[Preparation of sequences](#) for multiple alignment and tree-building, using
the [MABL](#) web site,
the [MAFFT](#) web site, or
[MEGA5](#) bioinformatics software

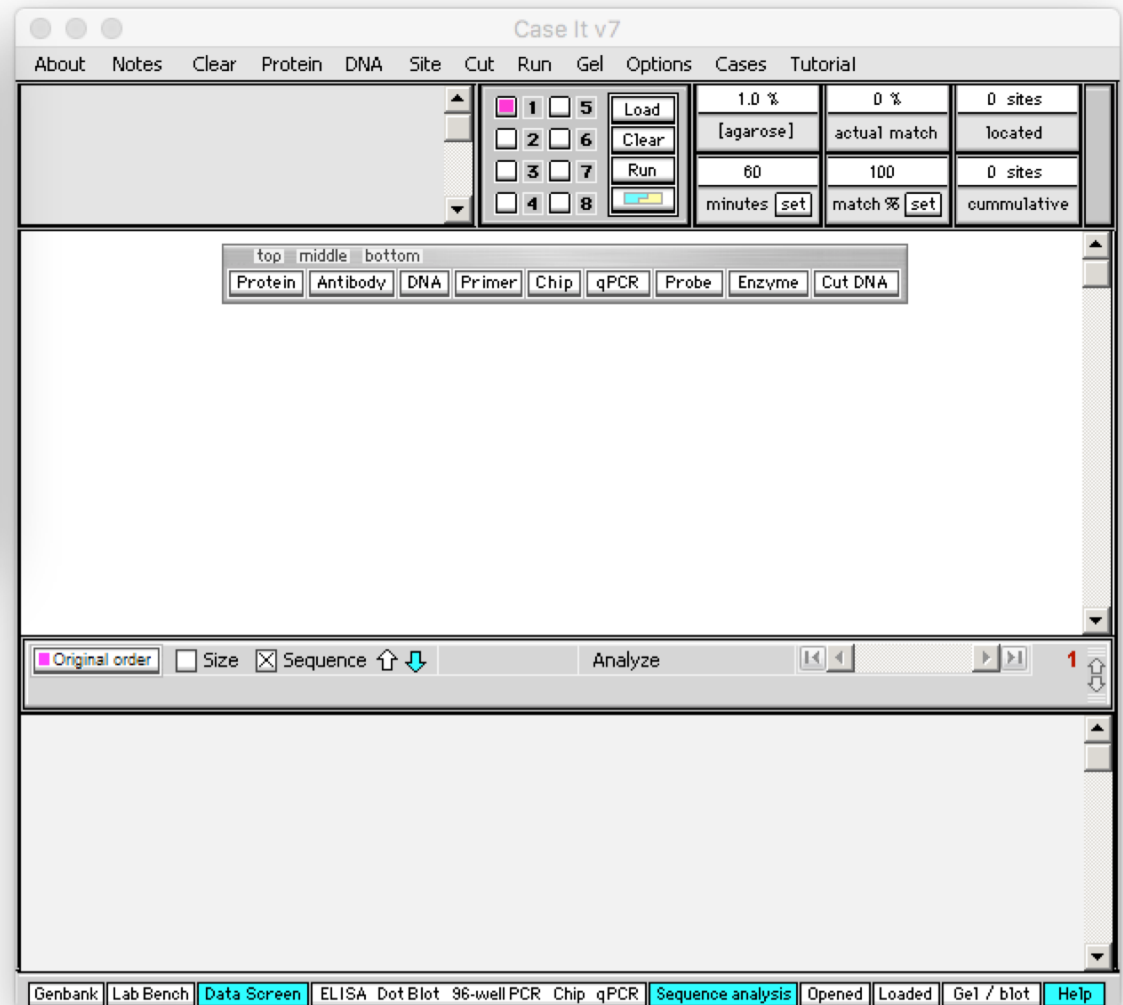
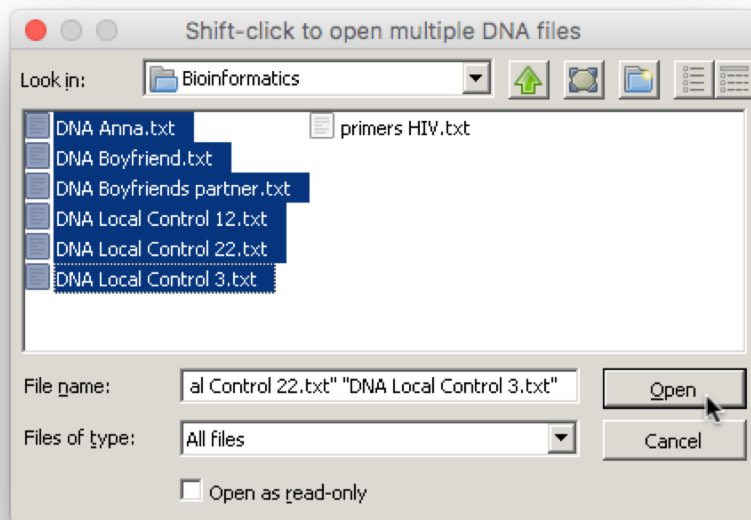
[Blasting](#) DNA and protein sequences

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.



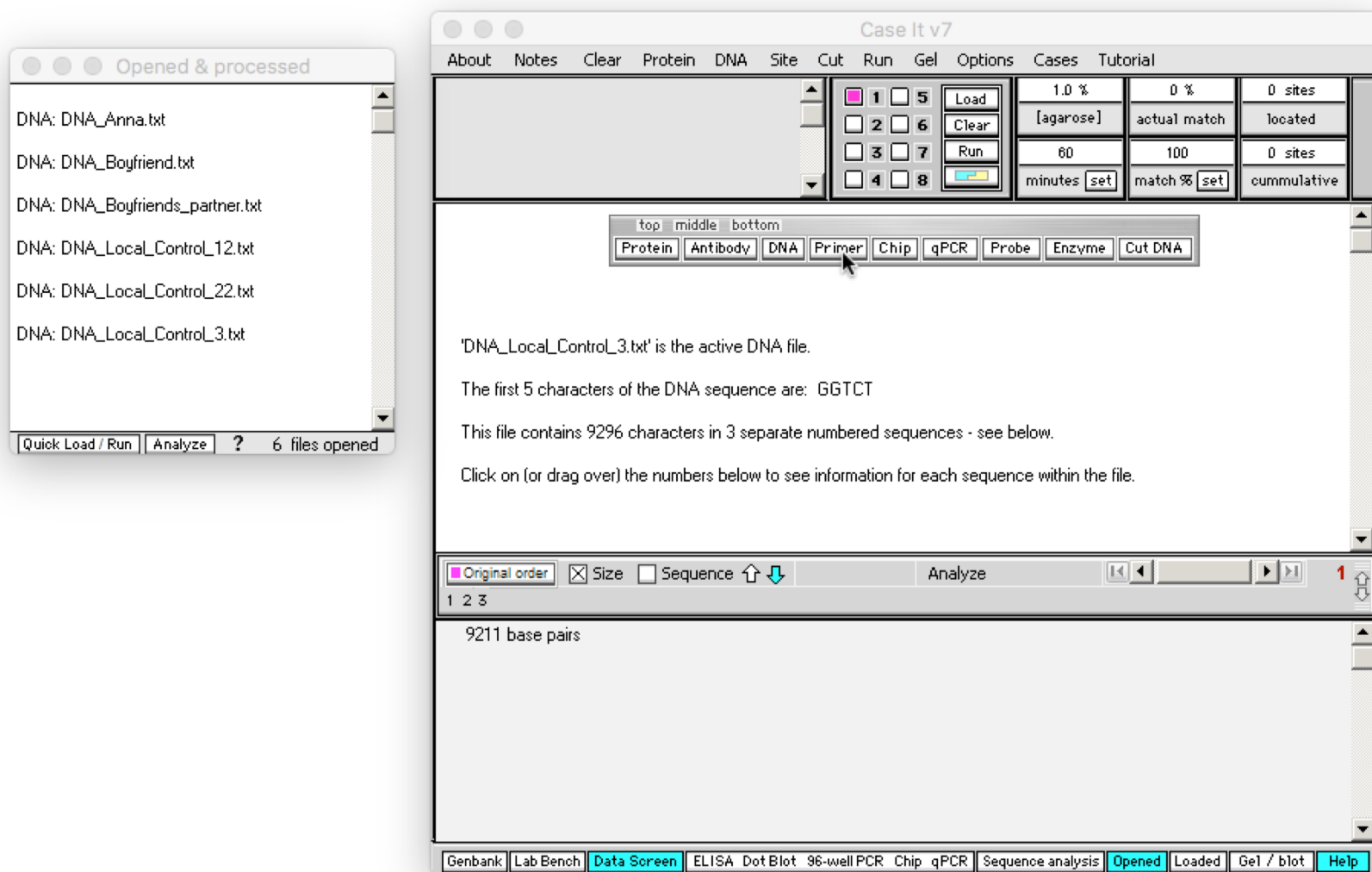
[Back to beginning](#)

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.



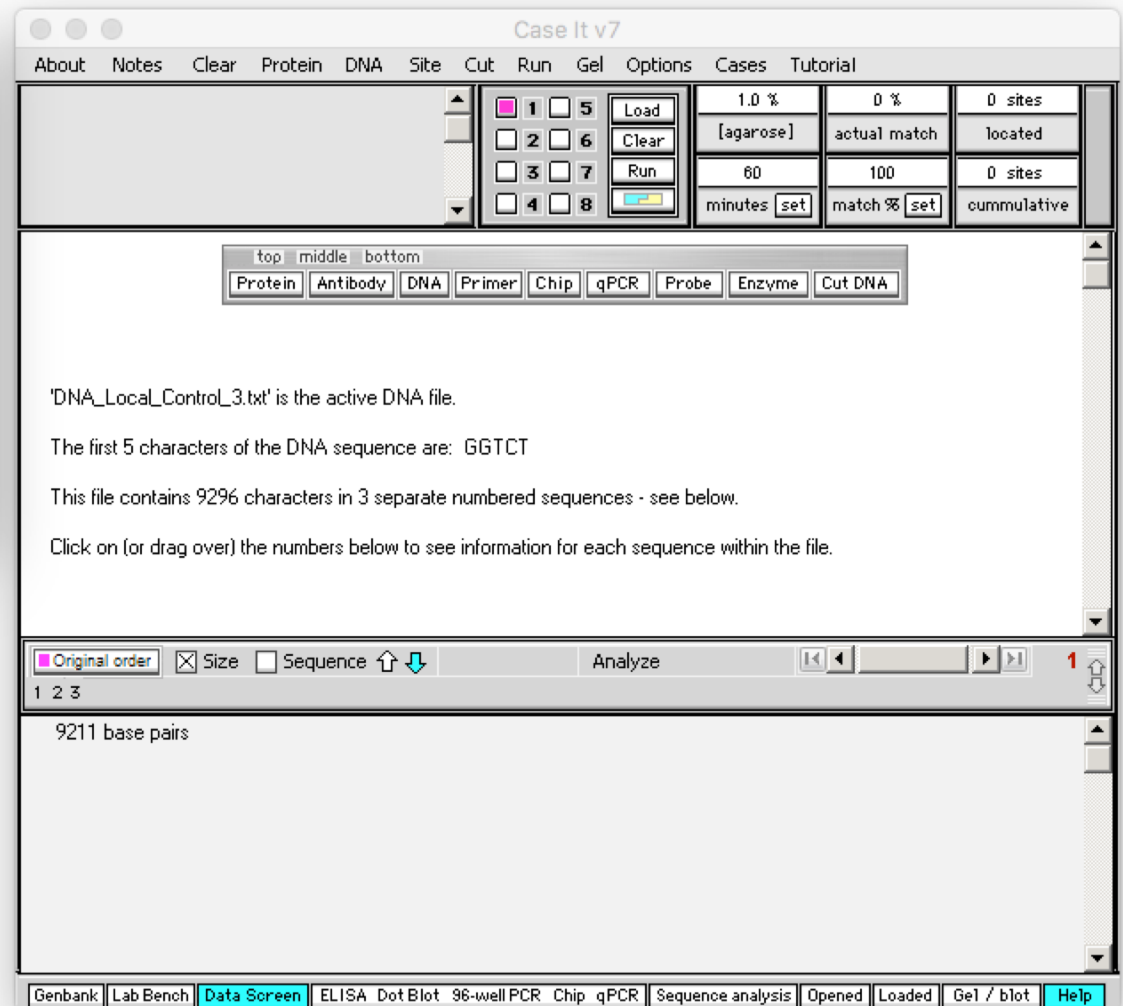
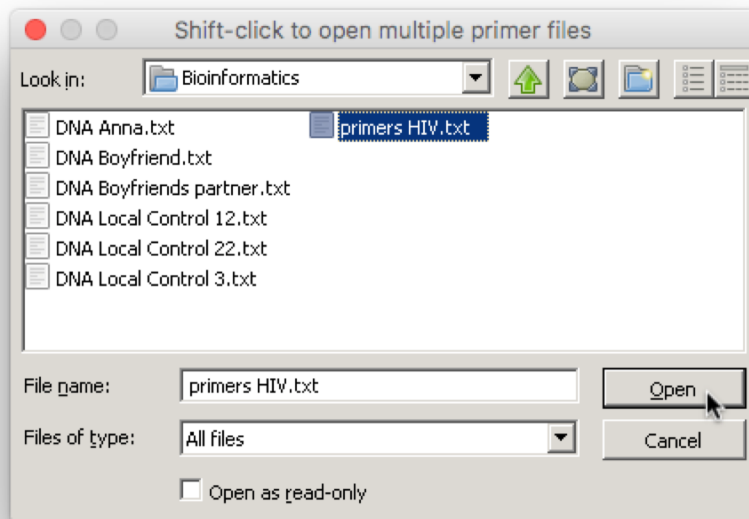
[Back to beginning](#)

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...



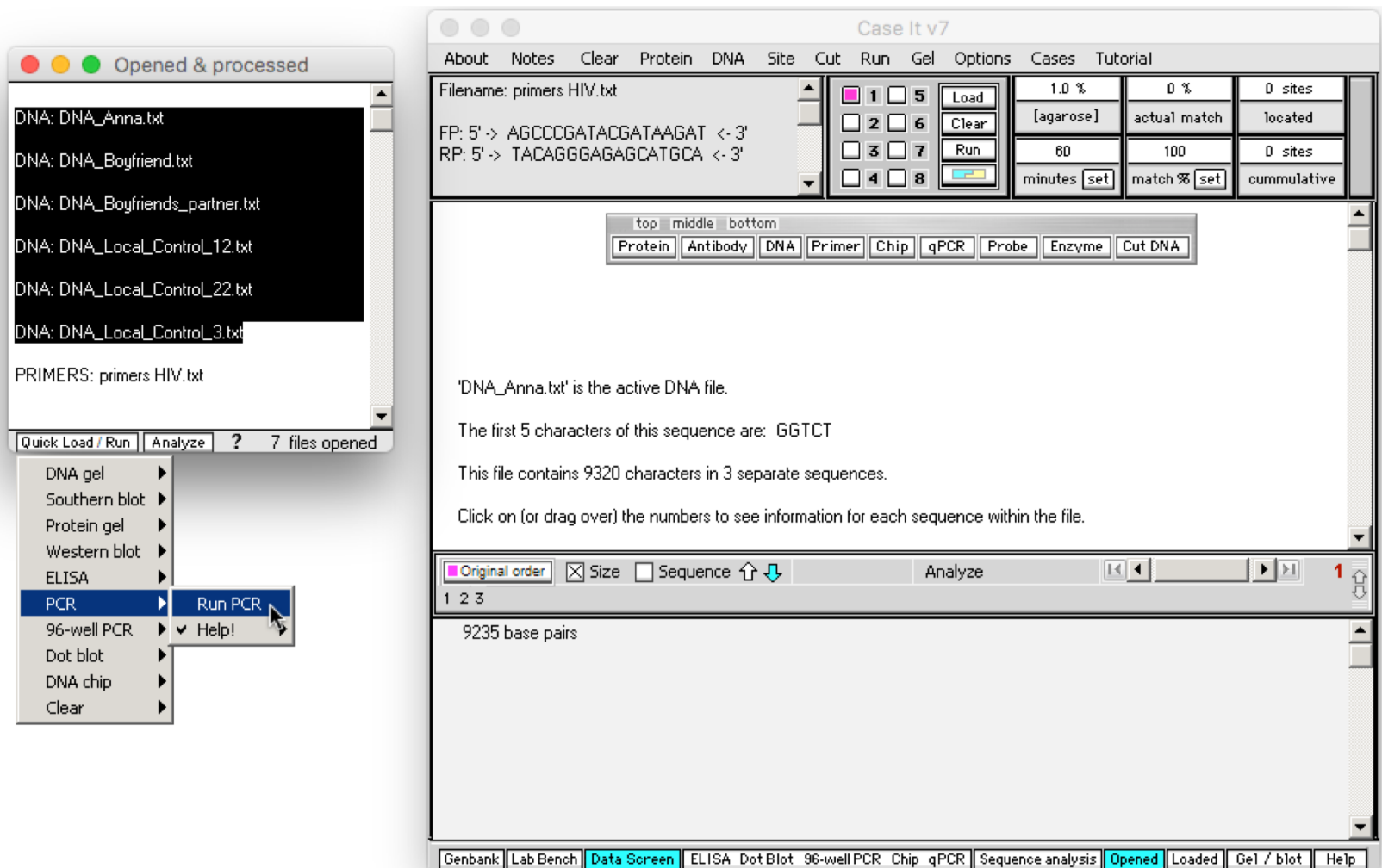
[Back to beginning](#)

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



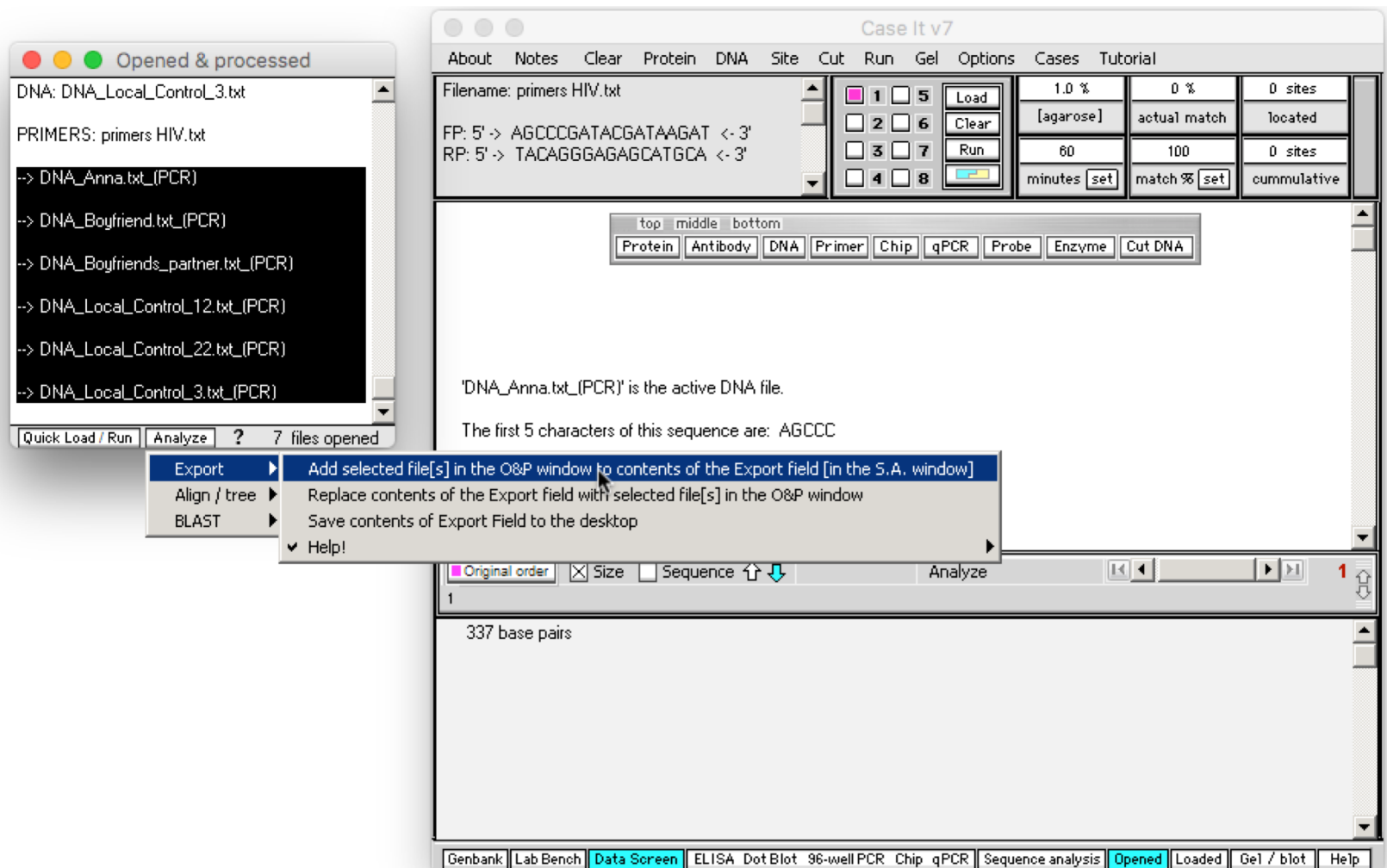
[Back to beginning](#)

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**.



[Back to beginning](#)

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]**.



[Back to beginning](#)

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.]

The screenshot displays the Case It v7 software interface, which is divided into three main windows:

- Opened & processed:** A list of files including DNA: DNA_Anna.txt, DNA: DNA_Boyfriend.txt, DNA: DNA_Boyfriends_partner.txt, DNA: DNA_Local_Control_12.txt, DNA: DNA_Local_Control_22.txt, DNA: DNA_Local_Control_3.txt, and PRIMERS: primers HIV.txt. A status bar at the bottom indicates "7 files opened".
- Case It v7 (Main Window):** The central workspace. The top menu includes About, Notes, Clear, Protein, DNA, Site, Cut, Run, Gel, Options, Cases, and Tutorial. The main area shows the filename "primers HIV.txt" and primer sequences:


```
FP: 5' -> AGCCCGATACGATAAGAT <- 3'
RP: 5' -> TACAGGGAGAGCATGCA <- 3'
```

 Below this are controls for loading, clearing, and running. A table shows parameters: 1.0% agarose, 0% actual match, 0 sites located, 60 minutes, 100% match, and 0 sites cumulative. A toolbar includes buttons for Protein, Antibody, DNA, Primer, Chip, qPCR, Probe, Enzyme, and Cut DNA. The main text area states: "DNA_Local_Control_3.txt(PCR)' is the active DNA file. The first 5 characters of this sequence are: AGCCCG. This file contains 355 characters in a single sequence." At the bottom, there are navigation and analysis controls, including an "Analyze" button and a sequence viewer showing the first few lines of the active sequence.
- Sequence analysis:** A window on the right for sequence analysis. It features a "Find FASTA" button, a search results field, and a list of sequences:


```
>DNA_Anna.txt(PCR)
AGCCCGATACGATAAGATGAGGTA
GTAATTAGATCTGCCAATTTACAG
ACAATGCTAAAATCATAATAGTACA
GCTGAATGCACCTGTAGAAATTA
TTGTACAAGACCCAACAATAACA
AGAAAAGGTATAAGTATAGGACCA
GGGAGAGCATT TTTATGCAACAGAT
AGAATAGTAGGAGATATAAGAAA
GCATATTGTAACATTAGTAGAGAA
AAATGGAAATAACTTTAAAACCTGG
TAGTTACAAAATTAAGAGAACAATT
TGTGAATAAAACAATAATCTTTAAT
CACTCCTCAGGAGGGGACCCAGAA
ATTGCATGCTCTCCCTGTA

|
|>DNA_Boyfriend.txt(PCR)
AGCCCGATACGATAAGATGAGGTA
GTAATTAGATCTGCCAATTTACAG
ACAATGCTAAAATCATAATAGTACA
GCTGAATGCATCTGTAGAAATTA
TTGTACAAGACCCAACAATAACA
AGAAAAGGTATACGTATAGGACCA
GGGAGAGCAGTTTATGCAGCAGAA
AAAATAATAGGAGATATAAGACGA
GCACATTGTAACATTAGTAGAGAA
AAATGGAAATAACTTTAAAACAGG
```

 Below the sequences are input fields for "Range-[1,19] Length-19", "Enter search sequence", and "Enter replacement sequence".

[Back to beginning](#)

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.]

The screenshot shows the Case It v7 software interface. On the left, the 'Opened & processed' window lists several DNA files, with 'DNA_Anna.txt(PCR)' selected. The 'Analyze' menu is open, showing a path: 'Analyze' -> 'Align / tree' -> 'from Export field' -> 'using MABL web site' -> 'Copy Export Field to clipboard and open MABL web site to 'one click' mode'. The main window displays the 'primers HIV.txt' file with sequence information and a table of parameters. The 'Sequence analysis' window on the right shows two DNA sequences: '>DNA_Anna.txt(PCR)' and '>DNA_Boyfriend.txt(PCR)'. The 'DNA_Anna.txt(PCR)' sequence is highlighted.

Case It v7

About Notes Clear Protein DNA Site Cut Run Gel Options Cases Tutorial

Filename: primers HIV.txt

FP: 5' -> AGCCCCGATACGATAAGAT <- 3'
 RP: 5' -> TACAGGGAGAGCATGCA <- 3'

1.0 %	0 %	0 sites
[agarose]	actual match	located
60	100	0 sites
minutes [set]	match % [set]	cummulative

top middle bottom

Protein Antibody DNA Primer Chip qPCR Probe Enzyme Cut DNA

'DNA_Local_Control_3.txt(PCR)' is the active DNA file.

The first 5 characters of this sequence are: AGCCC

This file contains 355 characters in a single sequence.

AGCCCCGATACGATAAGATGAGGTA
 ACCATAATAGTACAGCTAAATACATCTGTAAACAATTAATTGTACAAGACCTGGCAACAATACAAGAAAAAGTATAACTATGG
 GACCCGGGAAAGTATTTTATGCAGGAGAAATAATAGGAGATATAAGACAAGCACATTGTAACCTTAGTAGAACAGCATGGA
 ATGACACTTTAGAACAGATAGTTGGAAAAATACAAGAACAAATTTGGGAATAAAACAATAGTCTTTAATCACTCCTCAGGAGG
 GGACCCAGAAATTGCATGCTCTCCCTGTA

Genbank Lab Bench Data Screen ELISA Dot Blot 96-well PCR Chip qPCR Sequence analysis Opened Loaded Gel / blot Help

Sequence analysis

Options Find FASTA

Search results field

>DNA_Anna.txt(PCR)

AGCCCCGATACGATAAGATGAGGTA
 GTAATTAGATCTGCCAATTTACAG
 ACAATGCTAAATCATAATAGTACA
 GCTGAATGCACCTGTAGAAATTA
 TTGTACAAGACCCAACAATACA
 AGAAAAAGGTATAAGTATAGGACCA
 GGGAGAGCATTATGCAACAGAT
 AGAATAGTAGGAGATATAAGAAAA
 GCATATTGTAACATTAGTAGAGAA
 AAATGGAAATAACTTTAAACTGG
 TAGTTACAAAAATAGAGAACAAAT
 TGTGAATAAAAACAATAATCTTTAAT
 CACTCCTCAGGAGGGGACCCAGAA
 ATTGCATGCTCTCCCTGTA

>DNA_Boyfriend.txt(PCR)

AGCCCCGATACGATAAGATGAGGTA
 GTAATTAGATCTGCCAATTTACAG
 ACAATGCTAAATCATAATAGTACA
 GCTGAATGCATCTGTAGAAATTA
 TTGTACAAGACCCAACAATACA
 AGAAAAAGGTATAAGTATAGGACCA
 GGGAGAGCAGTTTATGCAGCAGAA
 AAAATAATAGGAGATATAAGACGA
 GCACATTGTAACATTAGTAGAGAA
 AAATGGAAATAACTTTAAACAGG

Range and length of selection

Enter search sequence

Enter replacement sequence

[Back to beginning](#)

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

The screenshot displays the MABL web interface in a browser window. The browser's address bar shows the URL `www.phylogeny.fr/simple_phylogeny.c`. The page features a navigation menu with options: Home, Phylogeny Analysis, Blast Explorer, Online Programs, Your Workspace, Documentation, and Downloads. A workflow diagram illustrates the process: Alignment MUSCLE → Curation Gblocks → Phylogeny PhyML → Tree Rendering TreeDyn. The 'One Click' mode is selected, and the '2. Data & Settings' tab is active. A text input field for the analysis name is present, followed by an upload section for FASTA, EMBL, or NEXUS files. A 'Browse...' button indicates 'No file selected'. Below this, a 'Paste' context menu is open over the input field, with 'Paste' highlighted. A 'Clear' button is located at the bottom right of the input area. On the left side, a sidebar shows a list of 'Opened & processed' files, including `DNA_Local_Control_3.txt`, `PRIMERS: primers HIV.txt`, and several PCR files. A 'Quick Load / Run | Analyze ? 7 files opened' button is visible at the bottom of the sidebar. A 'Back to beginning' link is located at the bottom left of the main content area.

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

The screenshot displays the MABL web site interface. On the left, a window titled "Opened & processed" lists several DNA files, including "DNA_Local_Control_3.txt", "PRIMERS: primers HIV.txt", and several PCR files. The main browser window shows the "Phylogeny.fr: 'One Click' Mode" page. The workflow diagram consists of four steps: "Alignment MUSCLE" → "Curation Gblocks" → "Phylogeny PhyML" → "Tree Rendering TreeDyn". The "Data & Settings" tab is active, showing a form for "Name of the analysis (optional):" and an "Upload your set of sequences" section. The "Or paste it here" section contains a text area with the following sequence data:

```
>DNA_Anna.txt_(PCR)
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCATAATAG
TACAGCTGAATGCACCTGTAGAAATTAATTGTACAAGACCCCAACAACAATACAAGAAAAGGTATAAGTAT
AGGACCAGGGAGAGCATTTTATGCAACAGATAGAAATAGTAGGAGATATAAGAAAAGCATATTGTAACATT
AGTAGAGAAAAATGGAATAAATACTTTAAAACAGGTAGTTACAAAATTAAGAGAACAATTTGTGAATAAAA
CAATAATCTTTAATCACTCCTCAGGAGGGGACCCAGAAAATGTCATGCTCTCCCTGTA

>DNA_Boyfriend.txt_(PCR)
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCATAATAG
TACAGCTGAATGCATCTGTAGAAATTAATTGTACAAGACCCCAACAACAATACAAGAAAAGGTATACGTAT
AGGACCAGGGAGAGCAGTTTATGCAACAGATAGAAATAGTAGGAGATATAAGAACGAGCACATTGTAACATT
AGTAGAGAAAAATGGAATAAATACTTTAAAACAGGTAGTTACAAAATTAAGAGAACAATTTGTGAATAAAA
CAATAATCTTTAATCACTCCTCAGGAGGGGACCCAGAAAATGTCATGCTCTCCCTGTA

>DNA_Boyfriends_partner.txt
AGCCCGATACGATAAGATGAGATAGTAATTAAATCTGCCAATTCACAGACAATGCTAAAATCATAATAG
```

Below the text area, there are instructions: "Maximum number of sequences is 200 for proteins and 200 for nucleic acids." and "Maximum length of sequences is 2000 for proteins and 6000 for nucleic acids." A checkbox is checked with the text "Use the Gblocks program to eliminate poorly aligned positions and divergent regions".

[Back to beginning](#)

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

Opened & processed

- DNA: DNA_Local_Control_3.txt
- PRIMERS: primers HIV.txt
- > DNA_Anna.txt(PCR)
- > DNA_Boyfriend.txt(PCR)
- > DNA_Boyfriends_partner.txt(PCR)
- > DNA_Local_Control_12.txt(PCR)
- > DNA_Local_Control_22.txt(PCR)
- > DNA_Local_Control_3.txt(PCR)

Quick Load / Run Analyze ? 7 files opened

Phylogeny.fr: "One Click" ...

www.phylogeny.fr/simple_phylogeny.c

Upload your set of sequences in FASTA, EMBL or NEXUS format from a file:

Browse... No file selected.

Or paste it here (load example of sequences)

```
TACAGCTGAATGCATCTGTAGAAATTAATTGTACAAGACCCAACAACACTATACAAGAAAAGGTATACGTAT
AGGACCAGGGGAGAGCAGTTTTATGCAGCAGAAAAAATAATAGGAGATATAAGACGAGCACATTGTAACATT
AGTAGAGAAAAATGGAATAATACCTTAAACAGGAGTGTACAAAATTAAGAGAACAATTTGTGAATAAAA
CAATAATCTTTAATCACTCCTCAGGAGGGGACCCAGAAATTCATGCTCTCCCTGTA

>DNA_Boyfriends_partner.txt
AGCCCGATACGATAAGATGAGATAGTAATTAATCTGCCAATTCACAGACAATGCTAAAAATCATAATAG
TACAGCTGAATGCATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAAGGTATACATAT
AGGACCAGGGGAGGGCATTATGCACAGGAGAAAAATAATAGGAGATATAAGACAAGCACATTGTAACATT
AGTGGAGAAAAATGGAATAATACCTTAAACAGGAGTGTACAAAATTAAGAGAACAATTTGGGAATAAAA
CAATAATCTTTAATCACTCCTCAGGAGGGGACCCAGAAATTCATGCTCTCCCTGTA

>DNA_Local_Control_12.txt_(
AGCCCGATACGATAAGATGAGGATAGTAATTAGATCTGCCGAAGTAGTAATTAGATCTGAAAAATTTACGG
ACAATGTTAAAACCAATAATAGACAGCTGAATGAATCTGTACAAAATTAATTGTACAAGACCCAACAACAA
TACAAGAAAAAGTATACATATAGCACCGGGGAGAGCATTATGCACAGGAGAAAAATAAGAGATATA
```

Clear

Maximum number of sequences is 200 for proteins and 200 for nucleic acids.
Maximum length of sequences is 2000 for proteins and 6000 for nucleic acids.

Use the Gblocks program to eliminate poorly aligned positions and divergent regions

To receive the results by e-mail, enter your address(es):

Submit

[Note:](#) beside sequences count and average length limit for the alignment stage there is also a limitation on the phylogeny stage (sequences_count*sequences_count*aligned_sequence_length=80000000) that will be checked once the alignment is done.

[Note:](#) usual bootstrapping procedure is replaced by a new confidence index that is much faster to compute.
See Anisimova M., Gascuel O. *Approximate likelihood ratio test for branches: A fast, accurate and powerful alternative.* Syst Biol. 2006, Aug;55(4):539-52. (PubMed)

Back to beginning

...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].

The screenshot displays the Phylogeny.fr website interface. On the left, a sidebar titled "Opened & processed" lists several DNA files and their corresponding PCR primers. The main content area shows the "One Click" Mode workflow: Alignment (MUSCLE) → Curation (Gblocks) → Phylogeny (PhyML) → Tree Rendering (TreeDyn). Below this, a progress bar indicates the current step is "5. Phylogeny". The "Tree Rendering results" section shows a phylogenetic tree with bootstrap values: 0.94, 0.86, 0.028, and 0.05. The tree includes sequences from DNA_Boyfriends_partner.txt, DNA_Boyfriend.txt_PCR, DNA_Anna.txt_PCR, DNA_Local_Control_3.txt_P, DNA_Local_Con, and DNA_Local_Control_22.txt. A "Back to beginning" link is located at the bottom left.

Opened & processed

- DNA: DNA_Local_Control_3.txt
- PRIMERS: primers HIV.txt
- > DNA_Anna.txt_PCR)
- > DNA_Boyfriend.txt_PCR)
- > DNA_Boyfriends_partner.txt_PCR)
- > DNA_Local_Control_12.txt_PCR)
- > DNA_Local_Control_22.txt_PCR)
- > DNA_Local_Control_3.txt_PCR)

Quick Load / Run Analyze ? 7 files opened

Phylogeny.fr: "One Click" ...

www.phylogeny.fr/simple_phylogeny.c

Méthodes et Algorithmes pour la Bio-informatique LIRMM

Information Génomique et Structurale

Home Phylogeny Analysis Blast Explorer Online Programs Your Workspace Documentation Downloads

"One Click" Mode

Alignment MUSCLE → Curation Gblocks → Phylogeny PhyML → Tree Rendering TreeDyn

1. Overview 2. Data & Settings 3. Alignment 4. Curation 5. Phylogeny 6. Tree Rendering

Tree Rendering results

0.94 0.86 0.028 0.05

DNA_Boyfriends_partner.txt
DNA_Boyfriend.txt_PCR
DNA_Anna.txt_PCR
DNA_Local_Control_3.txt_P
DNA_Local_Con
DNA_Local_Control_22.txt

Back to beginning

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.]

The screenshot displays the Case It v7 software interface. On the left, a sidebar titled 'Opened & processed' lists several DNA files, including 'DNA_Local_Control_3.txt'. The main window shows the 'Analyze' menu open, with the path: **Analyze** > **Align / tree** > **from Export field** > **using MAFFT web site** > **Copy Export Field to clipboard and open MAFFT web site**. The main window also displays sequence data for 'primers HIV.txt' and a list of analysis options like 'Protein', 'Antibody', 'DNA', etc. A secondary window titled 'Sequence analysis' is open on the right, showing search results for 'DNA_Anna.txt_(PCR)' and 'DNA_Boyfriend.txt_(PCR)'. At the bottom left, there is a blue link that says 'Back to beginning'.

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**.

The screenshot shows a web browser window with the URL <https://mafft.cbrc.jp/alignment/>. The page title is "MAFFT version 7" and the subtitle is "Multiple alignment program for amino acid or nucleotide sequences". The browser's address bar shows "LISTSERV 16.0 - DIRECT-L ..." and "MAFFT alignment and NJ / ...". The page content includes a "Download version" section with links for "Mac OS X", "Windows", "Linux", and "Source", and an "Online version" section with links for "Alignment", "mafft --add", "Merge", "Phylogeny", "Rough tree", "Merits / limitations", "Algorithms", "Tips", "Benchmarks", and "Feedback". The "Input:" section contains the text "Paste protein or DNA sequences in fasta format. [Example](#)" and a large text input field. A context menu is open over the input field, with the "Paste" option highlighted. The menu items include "Undo", "Cut", "Copy", "Paste", "Delete", "Select All", "Check Spelling", "Languages", "Block Element", "Inspect Element", "Random Agent Spoofer", and "Allow unusual symbols (Selenocysteine 'U', Inosine 'i', non-alphabetical)".

On the left side of the browser window, there is a sidebar with a list of files under the heading "Opened & processed":

- DNA: DNA_Local_Control_3.txt
- PRIMERS: primers HIV.txt
- > DNA_Anna.txt(PCR)
- > DNA_Boyfriend.txt(PCR)
- > DNA_Boysfriends_partner.txt(PCR)
- > DNA_Local_Control_12.txt(PCR)
- > DNA_Local_Control_22.txt(PCR)
- > DNA_Local_Control_3.txt(PCR)

Below the list are buttons for "Quick Load / Run", "Analyze", and "? 7 files opened".

At the bottom left of the image, there is a link: [Back to beginning](#)

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

Opened & processed

- DNA: DNA_Local_Control_3.txt
- PRIMERS: primers HIV.txt
- > DNA_Anna.txt(PCR)
- > DNA_Boyfriend.txt(PCR)
- > DNA_Boyfriends_partner.txt(PCR)
- > DNA_Local_Control_12.txt(PCR)
- > DNA_Local_Control_22.txt(PCR)
- > DNA_Local_Control_3.txt(PCR)

Quick Load / Run | Analyze | ? | 7 files opened

MAFFT alignment and NJ / ...

https://mafft.cbrc.jp/alignme

MAFFT version 7

Multiple alignment program for amino acid or nucleotide sequences

CBRC **AIST**

Multiple sequence alignment and NJ / UPGMA phylogeny

Input:
Paste protein or DNA sequences in fasta format. [Example](#)

```
>DNA_Anna.txt_(PCR)
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT
.....
>DNA_Boyfriend.txt_(PCR)
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT
.....
>DNA_Boyfriends_partner.txt
AGCCCGATACGATAAGATGAGATAGTAATTAATCTGCCAATTCACAGACAATGCTAAAATCAT
.....
>DNA_Local_Control_12.txt_(
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCGAAGTAGTAATTAGATCTGAAAATTT
.....
>DNA_Local_Control_22.txt_(
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGACAATTTCTCGGACAATGCTAGAACCAT
.....
```

or upload a plain text file: No file selected.

Use structural alignment(s)
 Allow unusual symbols (Selenocysteine "U", Inosine "i", non-alphabetical characters, etc.) [Help](#)

Genbank | Lab Ber

[Back to beginning](#)

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

The image shows a screenshot of a web browser displaying the MAFFT version 7 interface. On the left, a window titled "Opened & processed" lists several DNA files, including "DNA_Local_Control_3.txt". The browser window shows the URL "https://mafft.cbrc.jp/aligner/". The main content area is titled "MAFFT version 7" and includes a logo for CBRC and AIST. Below the title, there are several sections: "Download version" with links for Mac OS X, Windows, Linux, and Source; "Online version" with links for Alignment, mafft --add, Merge, Phylogeny, and Rough tree; "Merits / limitations", "Algorithms", "Tips", "Benchmarks", and "Feedback". The "Direction of nucleotide sequences" section has three radio button options, with "Same as input" selected. The "Output order" section has two radio button options, with "Aligned" selected. The "Notify when finished" section has an "Email address:" input field. At the bottom of this section, the "Submit" button is highlighted with a mouse cursor. Below this is the "Advanced settings" section, which includes a "Strategy:" section with a radio button for "Auto (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depends on data size) Updated" and a "Progressive methods" section with a radio button for "FFT-NS-1 (Very fast; recommended for >2,000 sequences; progressive method)".

Opened & processed

- DNA: DNA_Local_Control_3.txt
- PRIMERS: primers HIV.txt
- > DNA_Anna.txt(PCR)
- > DNA_Boyfriend.txt(PCR)
- > DNA_Boyfriends_partner.txt(PCR)
- > DNA_Local_Control_12.txt(PCR)
- > DNA_Local_Control_22.txt(PCR)
- > DNA_Local_Control_3.txt(PCR)

Quick Load / Run | Analyze | ? | 7 files opened

MAFFT alignment and NJ / ...

Filename: primers
FP: 5' -> AGCCCGATACG
RP: 5' -> TACACG

DNA_Local_Control_3.txt
The first 5 characters are
This file contains the following sequences

Original order
1

AGCCCGATACG
ACCATAATAGT
GACCGGGGAAA
ATGACACTTTA
GGACCCAGAAA

Genbank | Lab Bench

MAFFT version 7
Multiple alignment program for amino acid or nucleotide sequences

CBRC AIST

Download version
[Mac OS X](#)
[Windows](#)
[Linux](#)
[Source](#)

Online version
[Alignment](#)
[mafft --add](#)
[Merge](#)
[Phylogeny](#)
[Rough tree](#)

[Merits / limitations](#)
[Algorithms](#)
[Tips](#)
[Benchmarks](#)
[Feedback](#)

Direction of nucleotide sequences: [Help](#)

- Same as input
- Adjust direction according to the first sequence (accurate enough for most cases)
- Adjust direction according to the first sequence (only for highly divergent data; extremely slow)

Output order:

- Same as input
- Aligned

Notify when finished (optional; recommended when submitting large data):
Email address:

Submit Reset

Advanced settings

Strategy:
 Auto (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depends on data size) **Updated**

Progressive methods
 FFT-NS-1 (Very fast; recommended for >2,000 sequences; progressive method)

[Back to beginning](#)

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

Opened & processed

- DNA: DNA_Local_Control_3.txt
- PRIMERS: primers HIV.txt
- > DNA_Anna.txt(PCR)
- > DNA_Boyfriend.txt(PCR)
- > DNA_Boyfriends_partner.txt(PCR)
- > DNA_Local_Control_12.txt(PCR)
- > DNA_Local_Control_22.txt(PCR)
- > DNA_Local_Control_3.txt(PCR)

Quick Load / Run Analyze ? 7 files opened

Multiple sequence alignme... x +

https://mafft.cbrc.jp/alignment/ser... Search

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[LAST hits \(score>39\)](#) between the top sequence and the others.

[Open all plots](#)

[Clustal format](#) | [Fasta format](#) | [MAFFT result](#) | [View](#) | [Tree](#) | [Refine dataset](#) | [Return to home](#)

[View](#)

[Reformat](#) to GCG, PHYLIP, MSF, NEXUS, uppercase/lowercase, etc. with Readseq

[GUIDANCE2](#) computes the residue-wise confidence scores and extracts well-aligned residues.

[Refine dataset](#)

[Phylogenetic tree](#) **Visualization updated, 2016/Sep**

MAFFT-L-INS-i Result

CLUSTAL format alignment by MAFFT (v7.365)

```
DNA_Anna.txt_(P agcccgatacagataagatgaggtagtaattagatctgcc----
DNA_Boyfriend.t agcccgatacagataagatgaggtagtaattagatctgcc----
DNA_Boyfriends_ agcccgatacagataagatgaggtagtaattagatctgcc----
DNA_Local_Contr agcccgatacagataagatgaggtagtaattagatctgac----
DNA_Local_Contr agcccgatacagataagatgaggtagtaattagatctgccgaag
DNA_Local_Contr agcccgatacagataagatgaggtagtaattagatctgccgagg
*****

DNA_Anna.txt_(P aatttcacagacaatgctaaaatcataatagtagtacagctgaatg
DNA_Boyfriend.t aatttcacagacaatgctaaaatcataatagtagtacagctgaatg
DNA_Boyfriends_ aatttcacagacaatgctaaaatcataatagtagtacagctgaatg
```

[Back to beginning](#)

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

Opened & processed

- DNA: DNA_Local_Control_3.txt
- PRIMERS: primers HIV.txt
- > DNA_Anna.txt(PCR)
- > DNA_Boyfriend.txt(PCR)
- > DNA_Boyfriends_partner.txt(PCR)
- > DNA_Local_Control_12.txt(PCR)
- > DNA_Local_Control_22.txt(PCR)
- > DNA_Local_Control_3.txt(PCR)

Quick Load / Run Analyze ? 7 files opened

Multiple sequence alignme... x +

https://mafft.cbrc.jp/alignment/server/

Most Visited News UWRF Wikipedia Case It YouTube Prostate

LAST hits (score>39) between the top sequence and the others.

[Open all plots](#)

Be careful if there are **blue lines**. By default, MAFFT considers...

Clustal format | Fasta format | MAFFT result | View | Tree | Refine dataset | Return to home

NJ or UPGMA tree (β)

6 sequences, 358 total sites, 334 gap-free sites, 334 conserved sites

Go! Reset

Settings

Method:

- NJ - Conserved sites (334 bases)
- NJ - All gap-free sites (334 bases)
- Average linkage (UPGMA) - alignment scores (for up to 50,000 sequences)
- Minimum linkage - alignment scores (for up to 50,000 sequences)
- Memory-saving tree - alignment scores (for larger data)

Substitution model (valid when NJ is selected):

- Jukes-Cantor

Bootstrap (valid for NJ):

Back to beginning

...click **View tree on Phylo.io...**

Opened & processed

- DNA: DNA_Local_Control_3.txt
- PRIMERS: primers HIV.txt
- > DNA_Anna.txt(PCR)
- > DNA_Boyfriend.txt(PCR)
- > DNA_Boyfriends_partner.txt(PCR)
- > DNA_Local_Control_12.txt(PCR)
- > DNA_Local_Control_22.txt(PCR)
- > DNA_Local_Control_3.txt(PCR)

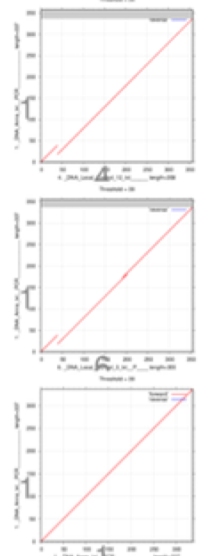
Quick Load / Run | Analyze ? 7 files opened

Multiple sequence alignment... x +

https://mafft.cbrc.jp/alignment/ser

LAST hits (score>39) between the top sequence and the others.

Open all plots



Be careful if there are blue lines. By default, MAFFT considers

Clustal format | Fasta format | MAFFT result | View | Tree | Refine dataset | Return to home

Result (Phylo.io 1.0.0) **Updated, 2016/Sep**

Phylo.io runs on any modern browser.

View tree on Phylo.io

Refine dataset on tree **Alpha testing, 2016/Aug**

Result (Archaeopteryx with Java plugin)

Uses Java plugin; no longer available on Chrome, Edge, etc.

View tree on Archaeopteryx (Signed; Forester 1.038)

View tree on Archaeopteryx (Unsigned; Forester 1.027; Try this if the signed version above does not work)

Refine dataset on tree

Result (Archaeopteryx without Java plugin)

0. Download and open [forester.jar](#), which starts [Archaeopteryx](#) as a standalone Java program.
1. On the Archaeopteryx window, select File → Read Tree from

[Back to beginning](#)

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

Opened & processed

DNA: DNA_Local_Control_3.txt

PRIMERS: primers HIV.txt

-> DNA_Anna.txt(PCR)

-> DNA_Boyfriend.txt(PCR)

-> DNA_Boyfriends_partner.txt(PCR)

-> DNA_Local_Control_12.txt(PCR)

-> DNA_Local_Control_22.txt(PCR)

-> DNA_Local_Control_3.txt(PCR)

Quick Load / Run | Analyze | ? | 7 files open

Phylo.io

Version: 1.0.k

View Compare

Tree:

```
(
1_DNA_Anna_txt_PCR
:0.0224,
2_DNA_Boyfriend_txt_PCR
:0.0176,(
3_DNA_Boyfriends_partner_
txt
:0.0167,(
5_DNA_Local_Control_22_tx
t
```

Render ▶

Settings

© Dessimoz Lab

Zoom:

4_DNA_Local_Control_12_txt

6_DNA_Local_Control_3_txt_P

5_DNA_Local_Control_22_txt

3_DNA_Boyfriends_partner_txt

1_DNA_Anna_txt_PCR

2_DNA_Boyfriend_txt_PCR

SVG

0.16

Find FASTA

GGTA
ACAG
TACA
TAA
TACA
ACCA
AGAT
AAAA
AGAA
CTGG
AATT
TAAT
AGAA

GGTA
ACAG
TACA
TAA
TACA
ACCA
AGAA
ACGA
AGAA
CAGG

[Back to beginning](#)

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 (MEGA5 is included with the Case It download). Click the **Analyze** button and select the menu commands below.

The screenshot displays the Case It v7 software interface. On the left, a window titled 'Opened & processed' lists several DNA files, with 'DNA_Anna.txt(PCR)' selected. The main window shows the 'primers HIV.txt' file with its sequence and primer information. A menu is open, showing the path: **Analyze** > **Align / tree** > **from Export field** > **using MEGA software** > **show alignment and tree**. A message box indicates that MEGA5.22 must be installed. On the right, a 'Sequence analysis' window shows the search results for 'DNA_Anna.txt(PCR)' and 'DNA_Boyfriend.txt(PCR)', displaying their respective DNA sequences.

[Back to beginning](#)

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.

The screenshot displays the Case It v7 software interface. A yellow message box is overlaid on the main window, providing instructions on waiting for the MEGA tree window to appear. The main window shows the 'Sequence analysis' panel with a list of DNA sequences and their corresponding PCR products. The 'Message' box contains the following text:

Please wait for the MEGA tree window to appear, which may take some time depending on the sizes of the sequences you are aligning. For larger sequences, this process can take a minute or longer.

Do not attempt to build another tree as you are waiting. You can do other Case It operations, however, that do not involve multiple alignment and tree-building.

Note: When you are finished viewing alignments and trees, closing the main MEGA window will automatically close the other MEGA windows.

A button labeled "Do not show this message again" is visible at the bottom of the message box.

The 'Sequence analysis' panel shows the following options and results:

- Options: Find FASTA
- Search results field: >DNA_Anna.txt_(PCR)
- AGCCCGATACGATAAGATGAGGTA
GTAATTAGATCTGCCAATTCACAG
ACAATGCTAAAATCATAATAGTACA
GCTGAATGCACCTGTAGAAATTAA
TTGTACAAGACCCCAACAATACA
AGAAAAGGTATAAGTATAGGACCA
GGGAGAGCATT T TATGCAACAGAT
AGAATAGTAGGAGATATAAGAAAA
GCATATTGTAACATTAGTAGAGAA
AAATGGAATAACTTTAAAACAGG
TAGTTACAAAATTAAGAGAACAAAT
TGTGAATAAAAACAATAATCTTTAAT
CACTCCTCAGGAGGGGACCCAGAA
ATTGCATGCTCTCCCTGTA
- >DNA_Boyfriend.txt_(PCR)
- AGCCCGATACGATAAGATGAGGTA
GTAATTAGATCTGCCAATTCACAG
ACAATGCTAAAATCATAATAGTACA
GCTGAATGCATCTGTAGAAATTAA
TTGTACAAGACCCCAACAATACA
AGAAAAGGTATAAGTATAGGACCA
GGGAGAGCAGTTTATGCAGCAGAA
AAAATAATAGGAGATATAAGACGA
GCACATTGTAACATTAGTAGAGAA
AAATGGAATAACTTTAAAACAGG
- Range and length of selection
- Enter search sequence
- Enter replacement sequence

The bottom of the interface shows a navigation bar with buttons for Genbank, Lab Bench, Data Screen, ELISA, Dot Blot, 96-well PCR, Chip, qPCR, Sequence analysis, Opened, Loaded, Gel / blot, and Help.

[Back to beginning](#)

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).

The screenshot displays the MEGA 5.05 software interface. A 'Confirm' dialog box is open in the center, asking if the user wants to download and install an update. The dialog includes the following text:

There is an update available. Would you like to download and install it now?
Update: http://update.megasoftware.net/MEGA6.06_setup.exe

NOTE: The "Ignore" button ignores this update and won't notify you till the next one is released.

The dialog has three buttons: 'Yes', 'No', and 'Ignore'. The 'Ignore' button is highlighted with a mouse cursor.

The background shows the MEGA 5.05 main window with a menu bar (Analysis, Help) and a toolbar. Below the toolbar is a navigation bar with links: 'st time User?', 'Tutorial', 'Examples', 'Citation', 'MEGA Web', 'Report a Bug', 'Updates?', 'Customize ToolBar', and 'Preferences'. The main window displays a sequence viewer for 'Site # 1' with the following sequence:

```
ACGGACAATACTAAA  
ACCATAAATAGTACAGCTAAATACATCTGTAACAATTAATTGTACAAGACCTGGCAACAATACAAGAAAAAGTAACTATGG  
GACCGGGGAAAGTATTTATGCAAGGAGAAATAATAGGAGATATAAGACAAGCAATTGTAACCTTAGTAGAACAGCATGGA  
ATGACACTTTAGAACAGATAGTTGGAAAAATACAAGAACAATTTGGGAATAAAACAATAGTCTTTAATCACTCCTCAGGAGG  
GGACCCAGAAATTGCATGCTCTCCCTGTA
```

On the right side, there is a 'Sequence analysis' window. It shows options for 'Search results field' and 'Find FASTA'. Two sequence files are listed:

- >DNA_Anna.tx_(PCR)
- >DNA_Boyfriend.tx_(PCR)

The 'DNA_Anna.tx_(PCR)' sequence is displayed in the main window's sequence viewer. The 'DNA_Boyfriend.tx_(PCR)' sequence is also visible in the 'Sequence analysis' window.

[Back to beginning](#)

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.

The screenshot displays the MEGA5 software interface. The main window, titled "M5: Alignment Explorer (out.fasta)", shows a multiple sequence alignment of six DNA sequences. The sequences are:

- 1. DNA Anna.txt PCR
- 2. DNA Boyfriend.txt PCR
- 3. DNA Boyfriends partner.txt
- 4. DNA Local Control 22.txt
- 5. DNA Local Control 12.txt
- 6. DNA Local Control 3.txt P

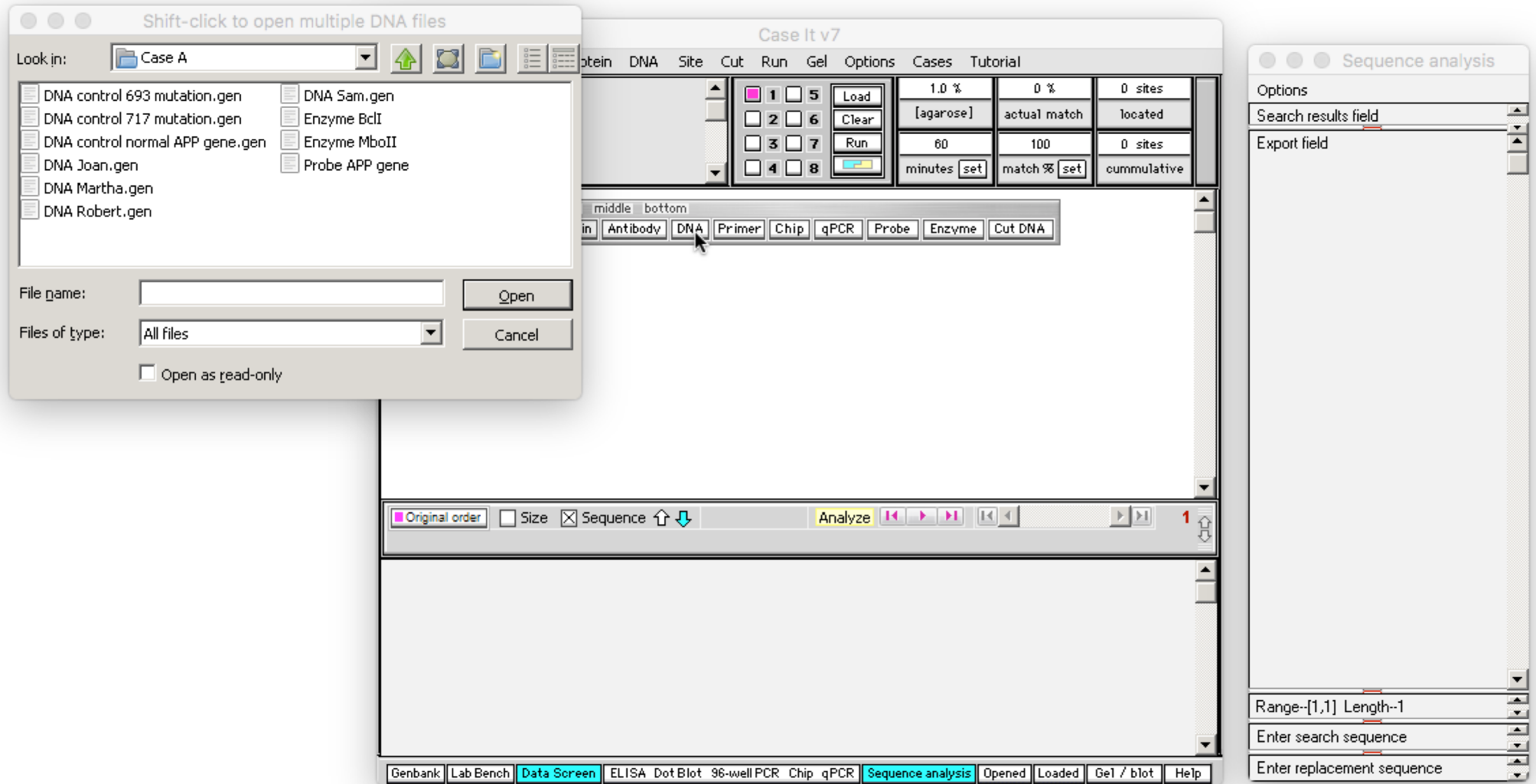
The alignment is color-coded by nucleotide: A (green), C (blue), G (red), and T (orange). Below the alignment, a phylogenetic tree is shown in the "M5: Tree Explorer (tree.nwk)" window. The tree is rooted and shows the following relationships:

- DNA Anna.txt PCR and DNA Boyfriend.txt PCR are sister taxa.
- DNA Boyfriends partner.txt is sister to the pair above.
- DNA Local Control 22.txt is sister to the group above.
- DNA Local Control 12.txt and DNA Local Control 3.txt P are sister taxa, and this pair is sister to the group above.

A scale bar of 0.01 substitutions per site is provided. The tree explorer window also shows the SBL value: SBL = 0.26514000. On the right side, a "Sequence analysis" panel is visible, showing a search results field with the text "NA_Anna.txt_(PCR)".

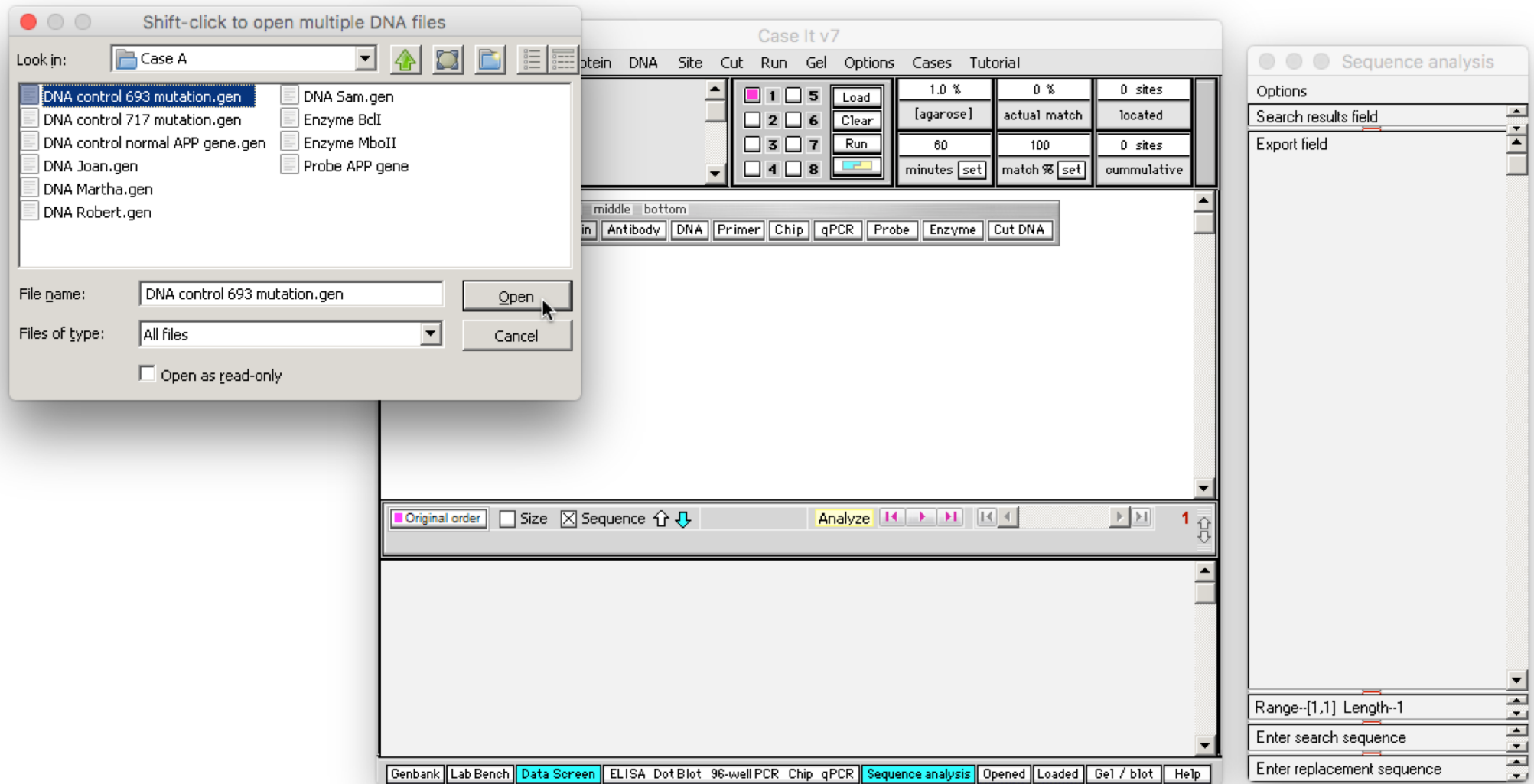
[Back to beginning](#)

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**.



[Back to beginning](#)

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...



[Back to beginning](#)

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.

The screenshot displays the Case It v7 software interface. On the left, a window titled "Opened & processed" shows the file name "DNA: DNA_control_693_mutation.gen". The main window, titled "Case It v7", has a menu bar with options: About, Notes, Clear, Protein, DNA, Site, Cut, Run, Gel, Options, Cases, Tutorial. Below the menu is a control panel with a grid of checkboxes (1-8) and buttons for "Load", "Clear", "Run", and "minutes". To the right of this panel are three columns of data: "1.0 %", "0 %", "0 sites"; "[agarose]", "actual match", "located"; and "60", "100", "0 sites". Below these are "minutes set", "match % set", and "cumulative". A central panel contains a "top middle bottom" selector and buttons for "Protein", "Antibody", "DNA", "Primer", "Chip", "qPCR", "Probe", "Enzyme", and "Cut DNA". The main text area states: "'DNA_control_693_mutation.gen' is the active DNA file. The first 5 characters of the DNA sequence are: tcaga. This file contains 4556 characters in 2 separate numbered sequences - see below. Click on (or drag over) the numbers below to see information for each sequence within the file." At the bottom of the main window is a divider bar with checkboxes for "Original order", "Size", and "Sequence" (checked), and a blue arrow pointing down. Below this bar are two sequence fields, with the first field containing the sequence: "tcagaagatcaatgctgcccgglttggcactgctcctgctggccgctggagcggctggggcgtggaggatccaccactgatggtaatgctggcctgctggctgaacccca gattgccatgttctggtggcagactgaacatgcacatgaatgtccagaatgggaagtggtgattcagatccatcaggaccacaaacctgcaltgataccaaggaaggcatc ctgcagataltgccaagaagctaccctgaactgcagatcaccatgtggtagaagccaaccaaccagtgaccatccagaactgggcaagcggggccgcaagcagtg gcaagaccatccccactttgtgattccctaccgctgcttagttggtgagttgtaagtgatgcccttctgctgacaagtgcaaatctacaccaggagaggatggatgt ttgcgaaactcatctcactggcacaccgctgccaagagagacatgcagtgagaagagtagccaacttgcatgactacggcatgttgcctgcccgtcggaaltgacaagttcc gggggtagagttgtgtgtgccactggctgaagaaagtgacaatgtggtctgctgatgcccaggaggatgactcggatgtcgtggggggggagcagacacagac tatgcagatgggagtgaaagcaaaagtagtagaagtagcagaggaggaagagtgctgaggtggaagaagaagaagccgatgatgacgaggacgatgaggatggt gatgaggtagaggaagggctgaggaaccctacgaagaagccacagagagaaccaccagcaltgccaccaccaccaccaccacagagctctggaagag gtggttcgagaggtgtgctgtaacaagccgagacggggcgtccgagccaatgatcaccgctggacttctgactgactgaaggaagtgccccaltctttacggc ggalgtggcggcaaccggaacaaccgtttgacacagaagagtagctgatggccgtgtgtggcagcgcaltctcaacagcagccagtagtaccctgatg". The bottom of the main window has a navigation bar with buttons: Genbank, Lab Bench, Data Screen, ELISA, Dot Blot, 96-well PCR, Chip, qPCR, Sequence analysis, Opened, Loaded, Gel / blot, Help. On the right, a "Sequence analysis" window is open, showing "Options", "Search results field", "Export field", "Range and length of selection", "Enter search sequence", and "Enter replacement sequence".

[Back to beginning](#)

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.

The screenshot displays the Case It v7 software interface. On the left, a window titled "Opened & processed" shows the file "DNA: DNA_control_693_mutation.gen". The main window, "Case It v7", has a menu bar with options: About, Notes, Clear, Protein, DNA, Site, Cut, Run, Gel, Options, Cases, Tutorial. Below the menu is a control panel with buttons for "Load", "Clear", "Run", and "Gel". To the right of these buttons are three columns of settings: "1.0 % [agarose]", "0 % actual match", and "0 sites located". Below these are "60 minutes" and "100 match %", each with a "set" button. A "cummulative" label is at the bottom right of this panel. The main text area contains the following text:

'DNA_control_693_mutation.gen' is the active DNA file.
The first 5 characters of the DNA sequence are: tcaga
This file contains 4556 characters in 2 separate numbered sequences - see below.
Click on (or drag over) the numbers below to see information for each sequence within the file.

Below this text is a sequence viewer with a toolbar showing "Original order", "Size", "Sequence", and "Analyze" buttons. The sequence text is displayed in a monospaced font. A context menu is open over the sequence text, with the following options:

- Copy selected text to clipboard including FASTA definition line, and open NCBI blast site
- Add selected text to existing contents of Export field
- Replace existing contents of Export field with selected text
- Copy selected text to clipboard
- Paste selected text into Search Field of Sequence Analysis window

At the bottom of the main window is a toolbar with buttons: Genbank, Lab Bench, Data Screen, ELISA, Dot Blot, 96-well PCR, Chip, qPCR, Sequence analysis, Opened, Loaded, Gel / blot, Help. On the right side, a "Sequence analysis" window is partially visible, showing "Options", "Search results field", and "Export field" sections.

[Back to beginning](#)

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.

The image shows a composite screenshot of a computer interface. On the left, a window titled "Opened & processed" displays the text "DNA: DNA_control_693_mutation.gen" and a status bar with "Quick Load / Run", "Analyze", "?", and "1 file opened". The main background is a web browser window showing the NCBI BLAST "Standard Nucleotide BLAST" page. The browser's address bar shows "https://blast.ncbi.nlm.nih.gov/Blast". The page header includes "NIH U.S. National Library of Medicine NCBI" and navigation links for "Home", "Recent Results", and "Saved Strategies". The "Enter Query Sequence" section is active, with a text input field containing "DNA_control_693_mutation.gen". A context menu is open over this field, listing options: "Undo", "Cut", "Copy", "Paste" (highlighted), "Delete", "Select All", and "Check Spelling Languages". To the right, a "Sequence analysis" sidebar is partially visible, showing "Options", "Search results field", and "Export field". At the bottom left, there is a blue hyperlink that reads "Back to beginning".

[Back to beginning](#)

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

Case It v7

About Notes Clear Protein DNA Site Cut Run Gel Options Cases Tutorial

1	5	Load	1.0 %	0 %	0 sites
2	6	Clear	[agarose]	actual match	located
3	7	Run	60	100	0 sites

Opened & processed

DNA: DNA_control_693_mutation.gen

Quick Load / Run Analyze ? 1 file opened

Sequence analysis

Options

Search results field

Export field

U.S. National Library of Medicine NCBI Sign in to

BLAST® » blastn suite Home Recent Results Saved Strategies

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#)

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#) [Query subrange](#) [?](#)

>DNA_control_693_mutation.gen
gattccctaccgctgcttagttggtgagtttgaagtgatgcccttctcgttccctgacaagtgcaaattc
ttacacc

From

To

Or, upload file No file selected. [?](#)

Job Title
Enter a descriptive title for your BLAST search [?](#)

Align two or more sequences [?](#)

Back to beginning

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

Case It v7

About Notes Clear Protein DNA Site Cut Run Gel Options Cases Tutorial

1	5	Load	1.0 %	0 %	0 sites
2	6	Clear	[agarose]	actual match	located
3	7	Run	80	100	0 sites

Opened & processed

DNA: DNA_control_693_mutation.gen

Quick Load / Run Analyze ? 1 file opened

Sequence analysis

Options

Search results field

Export field

Optional

Enter an Entrez query to limit search

Program Selection

Optimize for

- Highly similar sequences (megablast)
- More dissimilar sequences (discontiguous megablast)
- Somewhat similar sequences (blastn)

Choose a BLAST algorithm

BLAST Search database Nucleotide collection (nr/nt) using Blastn (Optimize for somewhat similar)

Show results in a new window

+ Algorithm parameters

BLAST is a registered trademark of the National Library of Medicine

Support center Mailing list YouTube

NCBI National Center for Biotechnology Information, U.S. National Library of Medicine

NATIONAL LIBRARY OF MEDICINE NIH

USA.gov

[Back to beginning](#)

After a few moments the BLAST results will appear.

The image shows a BLAST search interface. On the left, a window titled "Opened & processed" displays the file "DNA: DNA_controlL693_mutation.gen". Below it are buttons for "Quick Load / Run", "Analyze", and "?", and a status "1 file opened".

The main browser window shows the NCBI BLAST search results page. The address bar is "https://blast.ncbi.nlm.nih.gov/Blast". The search results are displayed as a grid of horizontal bars. Below the grid, the "Descriptions" section is visible, showing "Sequences producing significant alignments:" and "Select: All None Selected:0".

The "Alignments" section is expanded, showing a table of results:

	Description	Max score	Total score	Query cover	E value	Id
<input type="checkbox"/>	Human ORFeome Gateway entry vector pENTR223-APP, complete sequence	140	140	100%	5e-30	10
<input type="checkbox"/>	PREDICTED: Gorilla gorilla gorilla amyloid beta precursor protein (APP), transcript variant X1	140	140	100%	5e-30	10
<input type="checkbox"/>	PREDICTED: Gorilla gorilla gorilla amyloid beta precursor protein (APP), transcript variant X1	140	140	100%	5e-30	10
<input type="checkbox"/>	PREDICTED: Gorilla gorilla gorilla amyloid beta precursor protein (APP), transcript variant X1	140	140	100%	5e-30	10

At the bottom left, there is a link "Back to beginning".

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.

The screenshot displays three windows from the Case It v7 software. The 'Opened & processed' window on the left lists several DNA files and primers. The main 'Case It v7' window in the center shows the 'primers HIV.txt' file selected, with its forward and reverse primer sequences. Below this, it indicates that 'DNA_Local_Control_3.txt (PCR)' is the active DNA file and shows the first five characters of the sequence: 'AGCCC'. A menu is open over the 'Analyze' button, with the option 'Copy Export field to clipboard and open NCBI NUCLEOTIDE blast site' selected. The 'Sequence analysis' window on the right shows the search results for 'DNA_Anna.txt (PCR)', displaying a long DNA sequence with a vertical bar indicating the search position.

Opened & processed

DNA: DNA_Anna.txt
DNA: DNA_Boyfriend.txt
DNA: DNA_Boyfriends_partner.txt
DNA: DNA_Local_Control_12.txt
DNA: DNA_Local_Control_22.txt
DNA: DNA_Local_Control_3.txt
PRIMERS: primers HIV.txt
--> DNA_Anna.txt (PCR)

Quick Load / Run Analyze ? 7 files opened

Export
Align / tree
BLAST
Copy Export field to clipboard and open NCBI NUCLEOTIDE blast site
Copy Export field to clipboard and open NCBI PROTEIN blast site
Help

Case It v7

About Notes Clear Protein DNA Site Cut Run Gel Options Cases Tutorial

Filename: primers HIV.txt

FP: 5' -> AGCCCGATACGATAAGAT <- 3'
RP: 5' -> TACAGGGAGAGCATGCA <- 3'

1.0 % 0 % 0 sites
[agarose] actual match located
60 100 0 sites
minutes set match % set cumulative

top middle bottom
Protein Antibody DNA Primer Chip qPCR Probe Enzyme Cut DNA

'DNA_Local_Control_3.txt (PCR)' is the active DNA file.

The first 5 characters of this sequence are: AGCCC

This file contains 355 characters in a single sequence.

Analyze

AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCGAGGTAGTAATTAGATCTGAAAAATTCACGGACAATACTAAA
ACCATAATAGTACAGCTAAATACATCTGTAACAATTAATTGTACAAGACCTGGCAACAATACAAGAAAAAGTATAACTATGG
GACCGGGGAAAGTATTTTATGCAGGAGAAATAATAGGAGATATAAGACAAGCATTGTAACCTTAGTAGAACAGCATGGA
ATGACACTTTAGAACAGATAGTTGGAAAAATACAAGAACAATTTGGGAATAAAAAAATAGTCTTTAATCACTCCTCAGGAGG
GGACCCAGAAATTCATGCTCTCCCTGTA

Genbank Lab Bench Data Screen ELISA Dot Blot 96-well PCR Chip qPCR Sequence analysis Opened Loaded Gel / blot Help

Sequence analysis

Options Find FASTA

Search results field

>DNA_Anna.txt (PCR)

AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTTACAG
ACAATGCTAAAATCATAATAGTACA
GCTGAATGCACCTGTAGAAATTA
TTGTACAAGACCCAACAATAACA
AGAAAAAGGTATAAGTATAGGACCA
GGGAGAGCATTTTATGCAACAGAT
AGAATAGTAGGAGATATAAGAAAA
GCATATTGTAACATTAGTAGAGAA
AAATGGAAATAACTTTAAAACTGG
TAGTTACAAAATTAAGAGAACAATT
TGTGAATAAAAACAATAATCTTTAAT
CACTCCTCAGGAGGGGACCCAGAA
ATTGCATGCTCTCCCTGTA

|

>DNA_Boyfriend.txt (PCR)

AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTTACAG
ACAATGCTAAAATCATAATAGTACA
GCTGAATGCATCTGTAGAAATTA
TTGTACAAGACCCAACAATAACA
AGAAAAAGGTATACGTATAGGACCA
GGGAGAGCAGTTTATGCAGCAGAA
AAAAAATAGGAGATATAAGACGA
GCACATTGTAACATTAGTAGAGAA
AAATGGAAATAACTTTAAAAACAGG

Range and length of selection

Enter search sequence

Enter replacement sequence

[Back to beginning](#)

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

The image shows a multi-window interface for a BLAST search. On the left, a window titled "Opened & processed" lists several DNA files: DNA_Anna.txt, DNA_Boyfriend.txt, DNA_Boyfriends_partner.txt, DNA_Local_Control_12.txt, DNA_Local_Control_22.txt, DNA_Local_Control_3.txt, and PRIMERS: primers HIV.txt. Below the list are buttons for "Quick Load / Run", "Analyze", and "7 files opened".

The central window is the "BLAST Results" page for "6 sequences (DNA_Anna.txt_(PCR))". It features a navigation bar with "Home", "Recent Results", and "Save". Below this are links for "Edit and Resubmit", "Save Search Strategies", "Formatting options", and "Download". The "Results for:" section has a dropdown menu currently showing "1:lc|Query_95934 DNA_Anna.txt_(PCR)(337bp)". A mouse cursor is hovering over the selected item, which has opened a list of six options:

- 1:lc|Query_95934 DNA_Anna.txt_(PCR)(337bp)
- 2:lc|Query_95935 DNA_Boyfriend.txt_(PCR)(337bp)
- 3:lc|Query_95936 DNA_Boyfriends_partner.txt(337bp)
- 4:lc|Query_95937 DNA_Local_Control_12.txt_((358bp)
- 5:lc|Query_95938 DNA_Local_Control_22.txt_((337bp)
- 6:lc|Query_95939 DNA_Local_Control_3.txt_(P(355bp)

Below the dropdown is a table with columns for "RID", "Query ID", "Description", "Molecule type", and "Query Length". The "Query Length" is listed as 337. To the right, a table shows "Database Name", "Description", and "Program".

At the bottom of the BLAST Results page is a "Graphic Summary" section titled "Distribution of the top 100 Blast Hits on 100 subject sequence". It includes a "Color key for alignment scores" with four categories: <40 (black), 40-50 (blue), 50-80 (green), and 80-200 (magenta). Below the key is a horizontal bar chart for "Query" with markers at 1, 60, 120, 180, and 240. The chart shows several red horizontal bars at the bottom, indicating alignment scores.

On the far right, a vertical window shows a partial view of a DNA sequence: GTA, ACAG, ACA, TAA, ACA, CCA, GAT, AAA, GAA, CTGG, AAT, AAT, GAA.

At the bottom left of the image, there is a blue link that says "Back to beginning".

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.

The screenshot shows the Case It v7 software interface. On the left, a window titled "Opened & processed" lists several DNA files, with "DNA_Anna.txt(PCR)" selected. The main window, titled "Case It v7", shows the "primers HIV.txt" file with the following sequence:

```

FP: 5' -> AGCCCGATACGATAAGAT <- 3'
RP: 5' -> TACAGGGAGAGCATGCA <- 3'
  
```

The main window also displays the active DNA file, "DNA_Local_Control_3.txt(PCR)", and its first 5 characters: "AGCCC". A context menu is open over the sequence, with "BLAST" selected. The menu options include: Export, Align / tree, BLAST, Copy, Open, Save, Search, Replace, Clear, and Other. The BLAST option has a sub-menu with "Copy Export field to clipboard and open NCBI NUCLEOTIDE blast site" selected.

On the right, a "Sequence analysis" window shows the search results for "DNA_Anna.txt(PCR)". The results are displayed as a list of sequences, with "DNA_Anna.txt(PCR)" and "DNA_Boyfriend.txt(PCR)" visible. The "DNA_Anna.txt(PCR)" sequence is highlighted, and its full sequence is shown below:

```

AGCCCGATACGATAAGATGAGGTA
GTAATTAGATCTGCCAATTTACAG
ACAATGCTAAAATCATAATAGTACA
GCTGAATGCACCTGTAGAAATTA
TTGTACAAGACCCAACAATACA
AGAAAAGGTATAAGTATAGGACCA
GGGAGAGCATT TTTATGCAACAGAT
AGAATAGTAGGAGATATAAGAAA
GCATATTGTAACATTAGTAGAGAA
AAATGGAATAACTTTAAAACAGG
TAGTTACAAAATTAAGAGAACAATT
TGTGAATAAAACAATAATCTTTAAT
CACTCCTCAGGAGGGGACCCAGAA
ATTGCATGCTCTCCCTGTA
  
```

The "Sequence analysis" window also shows the "DNA_Boyfriend.txt(PCR)" sequence, which is identical to the one above. The window includes a "Find FASTA" button and a "Range" field set to "[1,355]" and "Length" set to "355".

[Back to beginning](#)

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.

The screenshot displays the Case It v7 software interface. On the left, a window titled "Opened & processed" lists several DNA files, with "DNA_Anna.txt (PCR)" selected. The main window, titled "Case It v7", shows the "primers HIV.txt" file loaded. The sequence editor displays the DNA sequence: "AGCCCGATACGATAAGATGAGGTAATAGATCTGCCGAGGTAGTAATTAGATCTGAAAATTCACGGACAATACTAAA...". A context menu is open over a highlighted portion of the sequence, with the option "Copy selected text to clipboard including FASTA definition line, and open NCBI blast site" selected. On the right, a "Sequence analysis" window shows search results for "DNA_Anna.txt (PCR)" and "DNA_Boyfriend.txt (PCR)", with the first few lines of the DNA sequence visible. The bottom of the Case It v7 window features a menu bar with options like "Genbank", "Lab Bench", "Data Screen", "ELISA", "Dot Blot", "96-well PCR", "Chip", "qPCR", "Sequence analysis", "Opened", "Loaded", "Gel / blot", and "Help".

[Back to beginning](#)

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.

The screenshot displays the Case It v7 software interface. On the left, a window titled "Opened & processed" lists files: "CHIP+: SNP cardiac disease.csv", "_1: Jonathan", "_2: SCD", and "_3: Low_risk". The main window shows a "DNA chip navi..." window with a grid of features. A context menu is open over a selected feature, offering options: "Copy selected probe sequence to clipboard and open NCBI nucleotide BLAST site", "Copy selected SNP ID to clipboard and open SNP database in default browser", and "Help".

The main data table is as follows:

	1	2	3	4	5	6
A	>rs7516560A 978	>rs17028318A 989	>rs9694082A 9794 CC	>rs11970286A 983	>rs2439383A 1634	>rs6765509A 5344 A
B	>rs7516560B 9223 TT	>rs17028318B 9956 GG	>rs9694082B 955	>rs11970286B 9752 CC	>rs2439383B 8947 TT	>rs6765509B 4768 G
C	>rs16932045A 1067	>rs3918242A 10231 CC	>rs11067228A 1127	>rs10948693A 1388	>rs6489615A 10467 CC	>rs6682594A 1266
			>rs11067228B 978 GG	>rs10948693B 8902 GG	>rs6489615B 1867	>rs6682594B 8795 GG
			>rs2928432A 392 A	>rs10757274A 10122 AA	>rs1912260A 8499 CC	>rs17721936A 9785 AA
F	>rs1564374B 3980 T	>rs2286690B 9025 GG	>rs2928432B 4908 G	>rs10757274B 1034	>rs1912260B 1104	>rs17721936B 1002
G	>rs7190509A 4232 C	>rs9998003A 1827	>rs4497735A 1530	>rs1043803A 4744 A	>rs8086719A 7944 AA	>rs10757278A 9099 AA
H	>rs7190509B 4523 T	>rs9998003B 10253 GG	>rs4497735B 9693 CC	>rs1043803B 4929 C	>rs8086719B 899	>rs10757278B 998

At the bottom, a navigation bar includes: "Genbank", "Lab Bench", "Data Screen", "ELISA", "Dot Blot", "96-well PCR", "Chip qPCR", "Sequence analysis", "Opened", "Loaded", "Gel / blot", and "Help".

[Back to beginning](#)