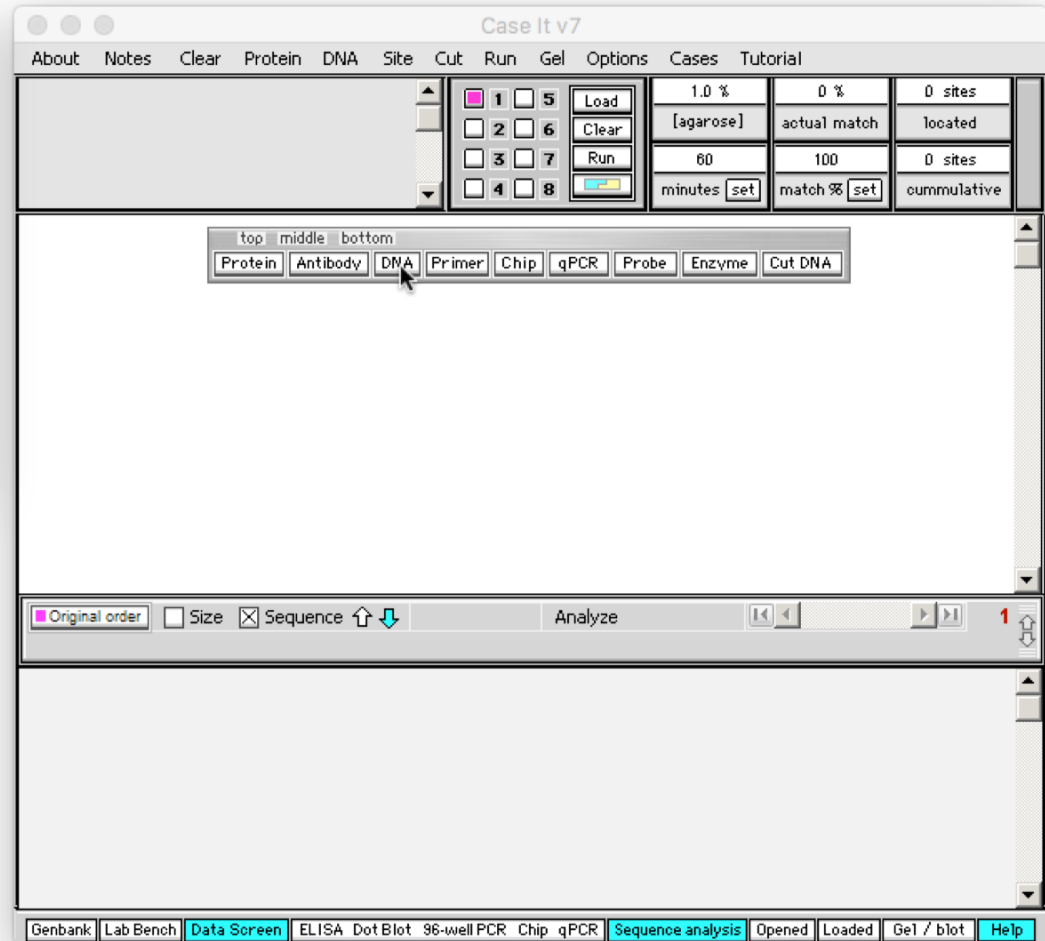
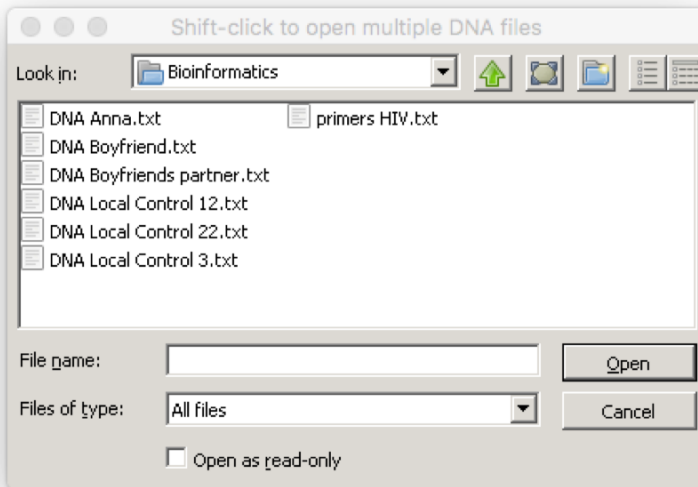


Tutorial for using Case It for bioinformatics analyses

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

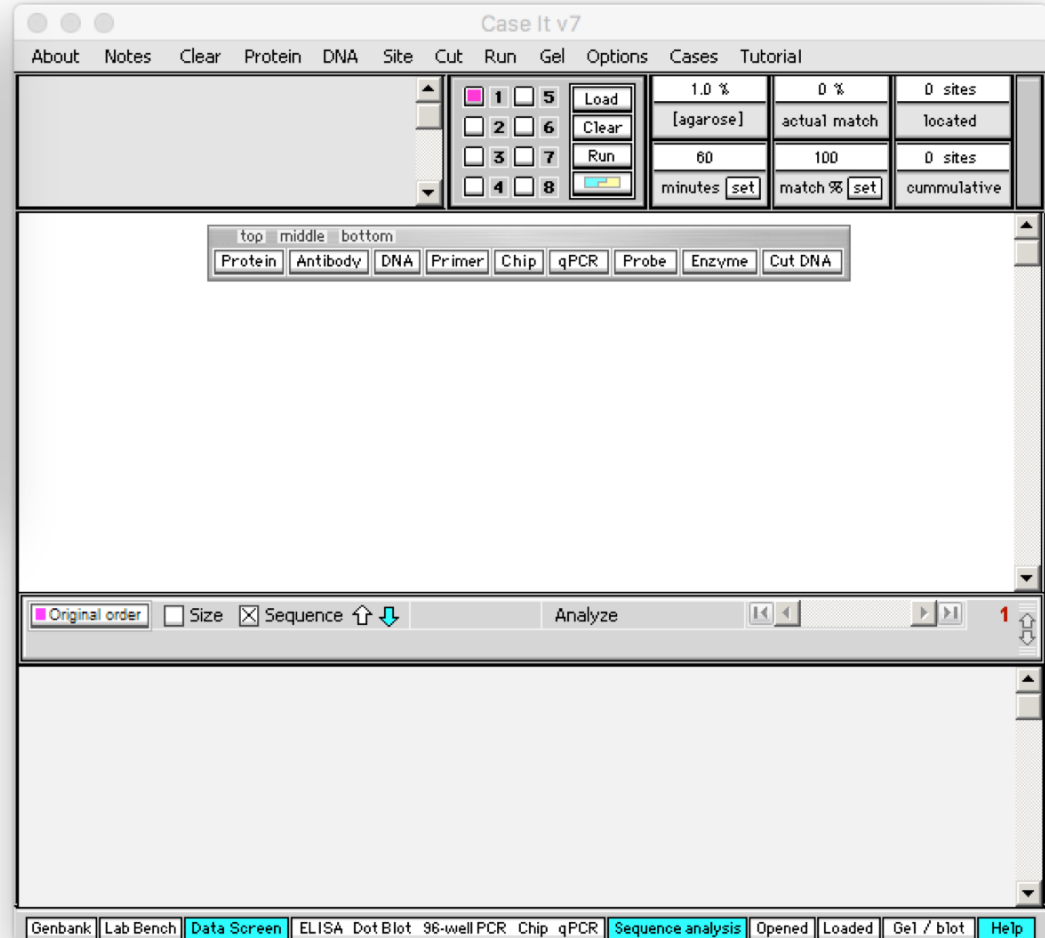
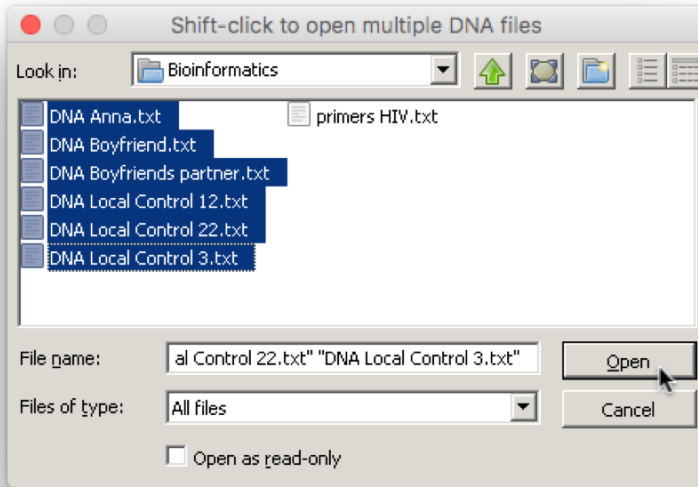
[Blasting](#) DNA and protein sequences

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.



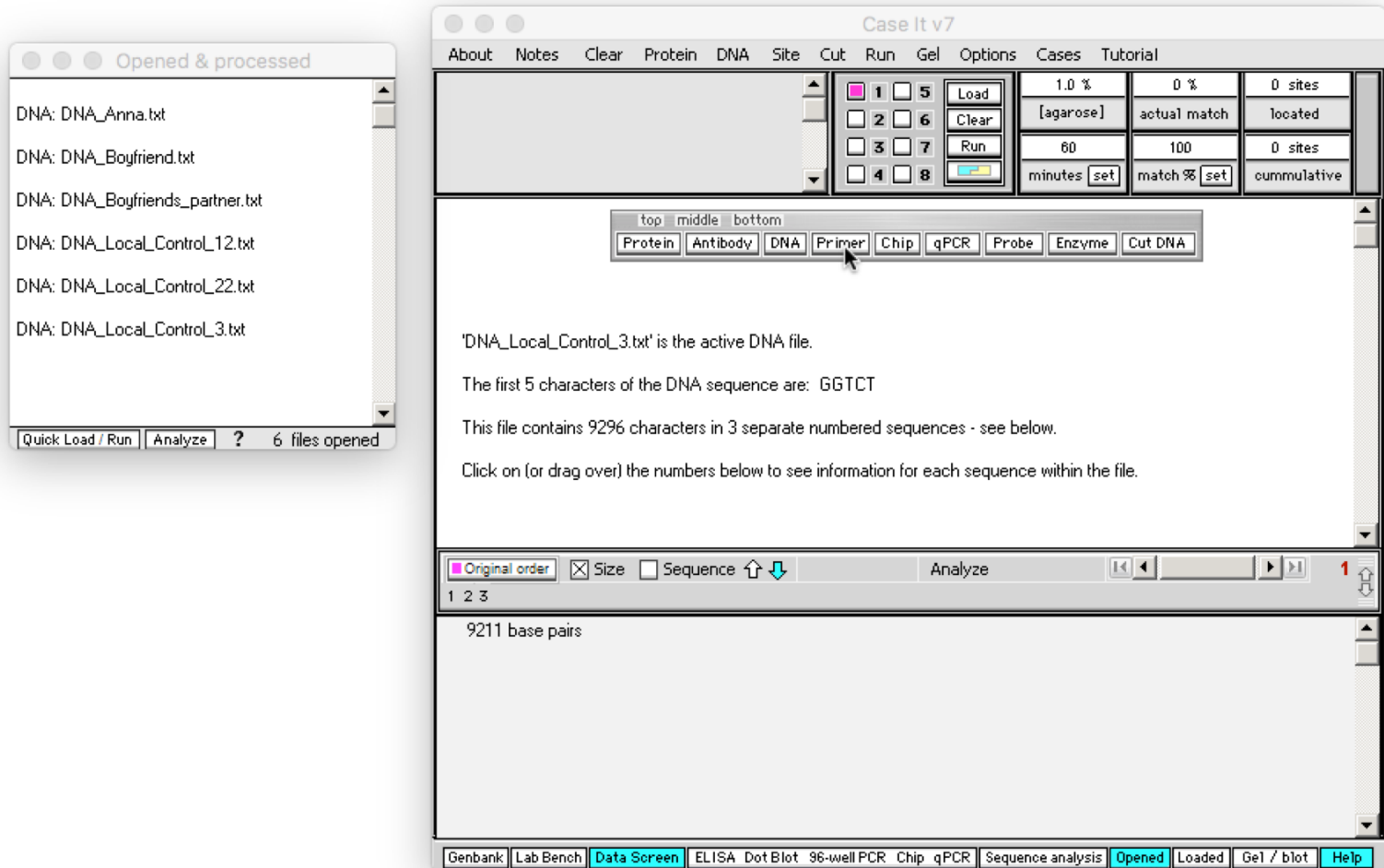
[Back to beginning](#)

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.



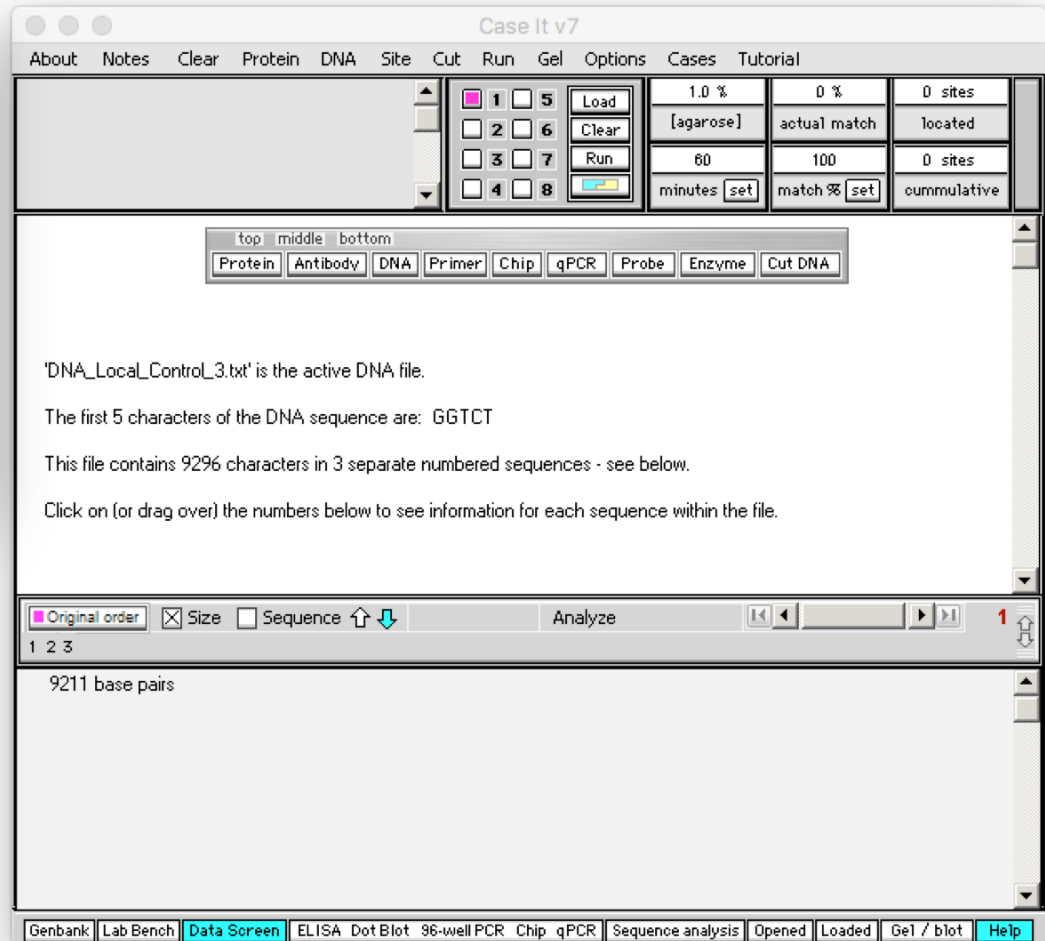
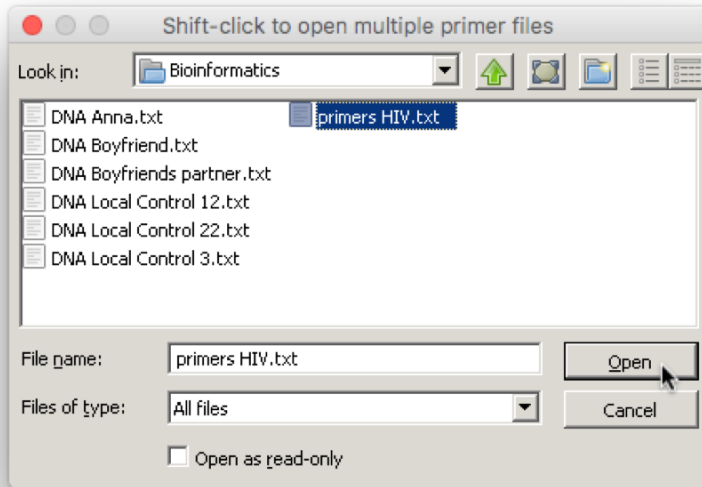
[Back to beginning](#)

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...



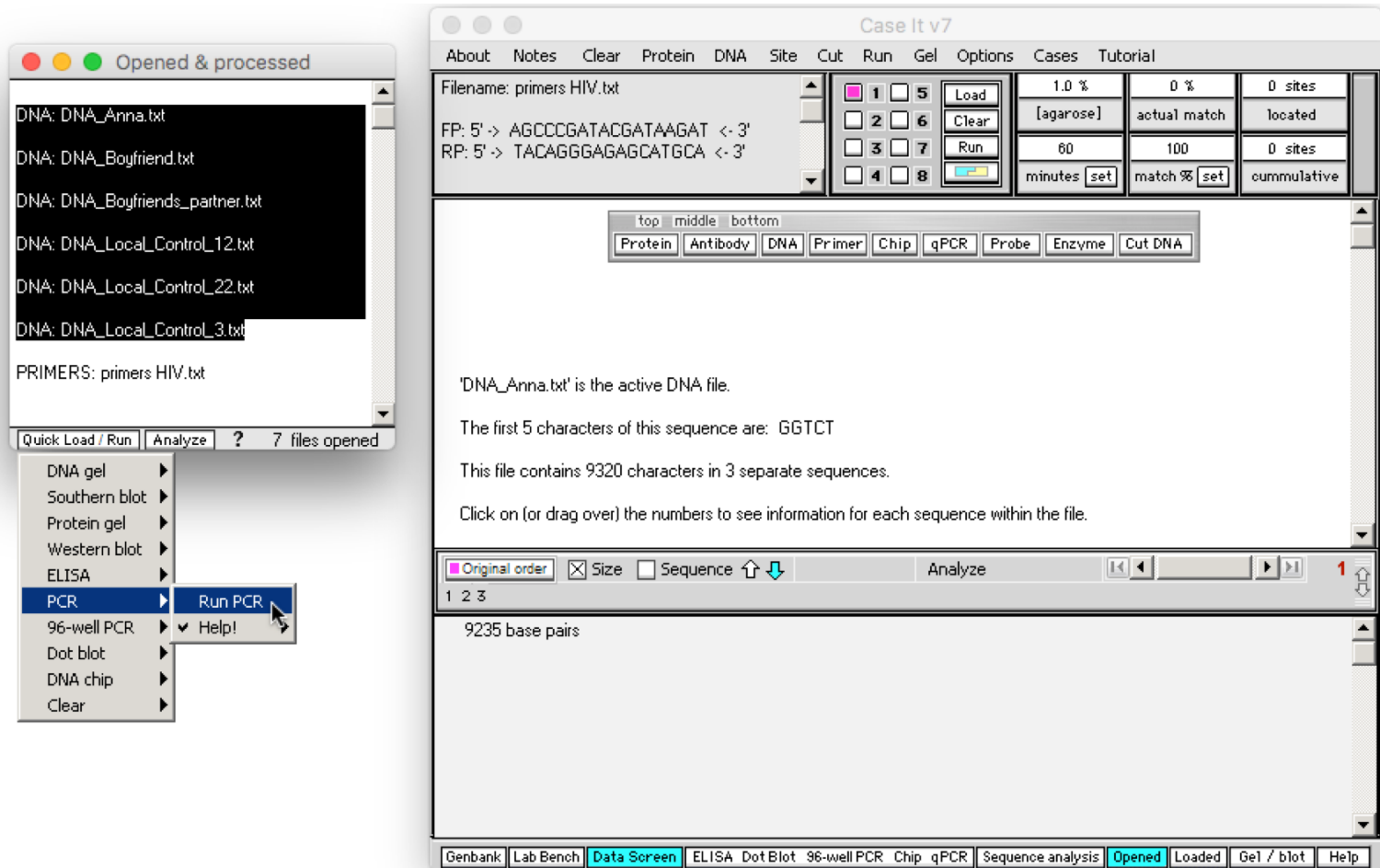
[Back to beginning](#)

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



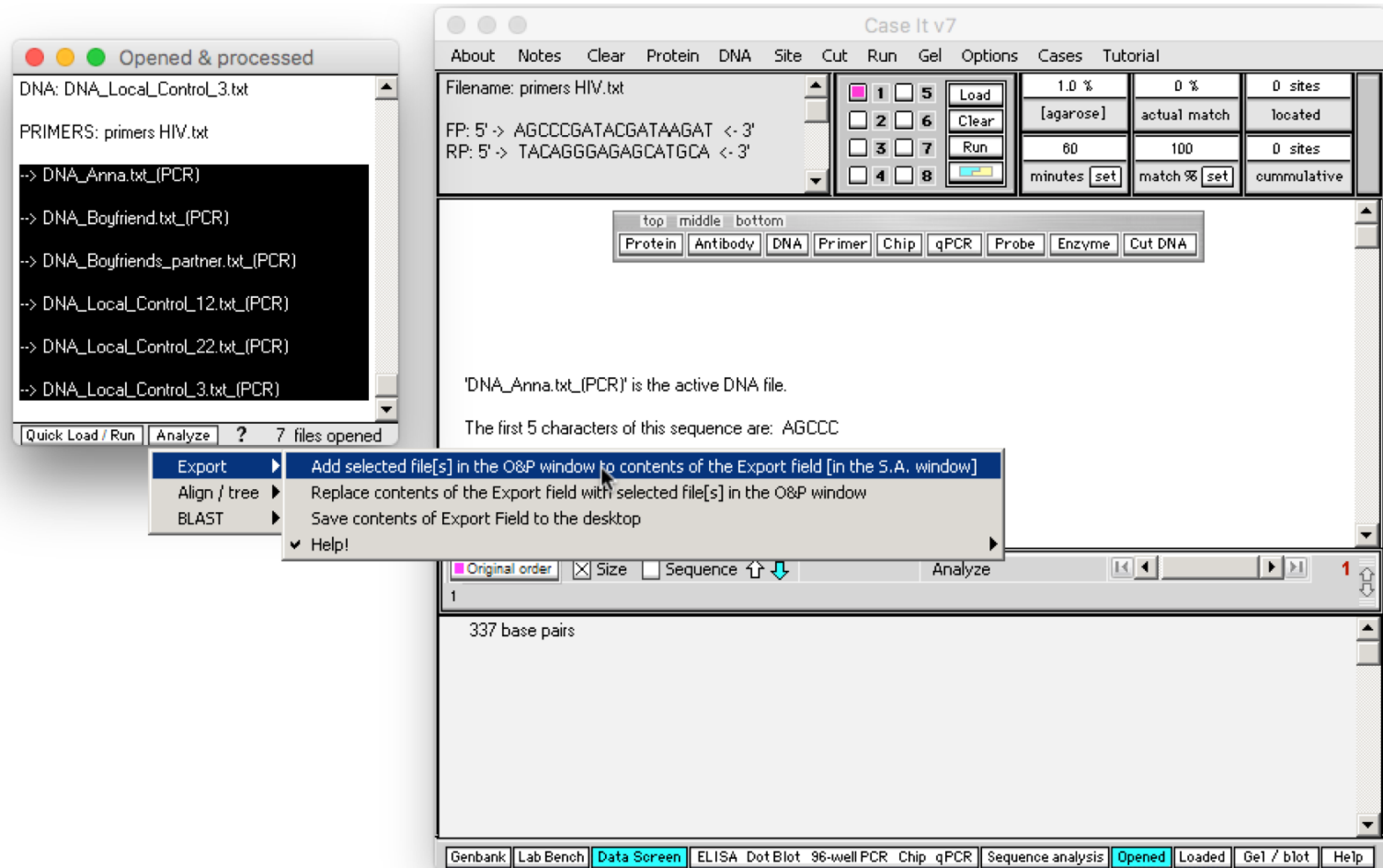
[Back to beginning](#)

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. On the left, a window titled "Opened & processed" lists several DNA files and primers, with "DNA_Anna.txt (PCR)" selected. The main window, titled "Case It v7", shows the "primers HIV.txt" file loaded. It displays the forward primer (FP) sequence 5'-AGCCCGATACGATAAGAT-3' and the reverse primer (RP) sequence 5'-TACAGGGAGAGCATGCA-3'. The main display area shows the active DNA file "DNA_Local_Control_3.txt (PCR)" and its sequence: AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCGAGGTAGTAATTAGATCTGAAAAATTCACGGACAATACTAAAACCATAGTACAGCTAAATACATCTGTAACAATTAATTGTACAAGACCTGGCAACAATACAAGAAAAAGTATAACTATGGACCCGGGAAAGTATTTTATGCAGGAGAAAAATAGGAGATATAAGACAAGCACATTGTAACCTTAGTAGAACAGCATGGAATGACACTTAGAACAGATAGTTGGAAAAATACAAGAACAAATTTGGGAATAAAAAACAATAGTCTTTAATCACTCCTCAGGAGGGACCCAGAAATTGCATGCTCTCCCTGTA. The bottom of the main window features a navigation bar with "Analyze" and "Sequence" options. On the right, the "Sequence analysis" window shows search results for "DNA_Anna.txt (PCR)" and "DNA_Boysfriend.txt (PCR)", displaying their respective FASTA sequences. A "Find FASTA" button is visible in the top right of this window.

[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 displays the Case It v7 software interface. On the left, the 'Opened & processed' window lists several DNA files, with 'DNA_Anna.txt (PCR)' selected. The main window shows the 'Analyze' menu 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 also displays the sequence 'DNA_Local_Control_3.txt (PCR)' and its first 5 characters 'AGCCC'. The right window shows the 'Sequence analysis' options, including 'Find FASTA' and 'Search results field'.

[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 title is "Méthodes et Algorithmes pour la Bio-informatique LIRMM". The navigation menu includes "Home", "Phylogeny Analysis", "Blast Explorer", "Online Programs", "Your Workspace", "Documentation", and "Downloads".

The main content area is titled "One Click" Mode and features a workflow diagram: `Alignment MUSCLE` → `Curation Gblocks` → `Phylogeny PhyML` → `Tree Rendering TreeDyn`. Below this, there are two tabs: "1. Overview" (selected) and "2. Data & Settings".

The "Overview" tab contains a form for "Name of the analysis (optional):" and a section for "Upload your set of sequences in FASTA, EMBL or NEXUS format from a file:" with a "Browse..." button and the text "No file selected." Below this is a section for "Or paste it here (load example of sequences)" with a large text input area. A context menu is open over this input area, showing options: "Undo", "Cut", "Copy", "Paste" (highlighted), "Delete", and "Select All". A "Clear" button is located at the bottom right of the input area.

On the left side, a separate window titled "Opened & processed" lists several files and their processing status, including `DNA: DNA_Local_Control_3.txt` and `PRIMERS: primers HIV.txt`. Below this list are buttons for "Quick Load / Run", "Analyze", and a status indicator "7 files opened".

At the bottom left of the main interface, there is a link labeled "Back to beginning".

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

About No

Filename: pri

FP: 5' -> AG

RP: 5' -> TA

'DNA_Loc

The first 5

This file c

Original ord

1

AGCCCGAT

ACCATAA7

GACCGGGE

ATGACACT

GGACCCAG

Genbank Lal

Phylogeny.fr: "One Click" ...

www.phylogeny.fr/simple_phylogeny.c

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

Name of the analysis (optional):

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)

```
>DNA_Anna.txt_(PCR)
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAAATCATAATAG
TACAGCTGAATGCACCTGTAGAAATTAATTGTACAAGACCCAAACAATACAAGAAAAGGTATAAGTAT
AGGACCAGGGAGAGCATTATGCAACAGATAGAAATAGTAGGAGATATAAGAAAAGCATATTGTAACATT
AGTAGAGAAAAATGGAATAACTTTAAAACAGCTAGTACAAAATTAAGAGAACAAATTTGTGAATAAAA
CAATAATCTTTAATCACTCCTCAGGAGGGGACCCAGAAATTCATGCTCTCCCTGTA

>DNA_Boyfriend.txt_(PCR)
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAAATCATAATAG
TACAGCTGAATGCACCTGTAGAAATTAATTGTACAAGACCCAAACAATACAAGAAAAGGTATAAGTAT
AGGACCAGGGAGAGCATTATGCAACAGATAGAAATAGTAGGAGATATAAGAAAAGCATATTGTAACATT
AGTAGAGAAAAATGGAATAACTTTAAAACAGCTAGTACAAAATTAAGAGAACAAATTTGTGAATAAAA
CAATAATCTTTAATCACTCCTCAGGAGGGGACCCAGAAATTCATGCTCTCCCTGTA

>DNA_Boyfriends_partner.txt
AGCCCGATACGATAAGATGAGATAGTAATTAATCTGCCAATTCACAGACAATGCTAAAAATCATAATAG
```

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

[Back to beginning](#)

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

The screenshot shows a web browser window at www.phylogeny.fr/simple_phylogeny.c. The page title is "Phylogeny.fr: 'One Click' ...". The browser's address bar and tabs are visible at the top.

On the left side, there is a sidebar titled "Opened & processed" with a list of files:

- 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)

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

The main content area of the browser window contains the following text:

Upload your set of sequences in FASTA, EMBL or NEXUS format from a file:
 No file selected.

Or paste it here ([load example of sequences](#))

```
TACAGCTGAATGCATCTGTAGAAATTAATTGTACAAGACCCAACTATACAAGAAAAGGTATACGTAT
AGGACCAGGGAGAGCAGTTTATGCAGCAGAAAAATAATAGGAGATATAAGACGAGCACATTGTAACATT
AGTAGAGAAAAATGGAATAAATCTTTAAAACAGGTAGTTACAAAATTAAGAGAACAAATTTGGGAATAAAA
CAATAATCTTTACTCACCCCTCAGGAGGGGACCCAGAAATTGCATGCTCTCCCTGTA

>DNA_Boyfriends_partner.txt
AGCCCCGATACGATAAGATGAGTAGTAATTTAAATCTGCCAATTCACAGACAATGCTAAAAATCATAATAG
TACAGCTGAATGCATCTGTAGAAATTAATTGTACAAGACCCAACTATACAAGAAAAGGTATACATAT
AGGACCAGGGAGGGCATTATGCAACAGGAGAAATAATAGGAGATATAAGACAGCACATTGTAACATT
ACTGGAGAAAATGGAAATAAATCTTTAAAACAGGTAGTTACAAAATTAAGAGAACAAATTTGGGAATAAAA
CAATAATCTTTAAATCACTCCTCAGGAGGGGACCCAGAAATTGCATGCTCTCCCTGTA

>DNA_Local_Control_12.txt_(
AGCCCCGATACGATAAGATGAGGTAGTAATTTAGATCTGCCGAAGTAGTAATTTAGATCTGAAAAATTCACGG
ACAATGTTAAAACATAATAGAGCAGCTGAATGAATCTGTACAAAATTAATTGTACAAGACCCAACTAA
TACAAGAAAAGTATACATATAGCACCGGGGAGAGCATTATGCAACAGGAGAAATAATAGAGATATA
```

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)**:

[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=8000000) 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)

At the bottom left, there is a link: [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].

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

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

0.05

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 list of files is shown under 'Opened & processed', including 'DNA: DNA_Local_Control_3.txt' and 'PRIMERS: primers HIV.txt'. The main window shows the 'primers HIV.txt' file with its sequence: 'FP: 5' -> AGCCCGATACGATAAGAT <- 3'' and 'RP: 5' -> TACAGGGAGAGCATGCA <- 3''. The 'Analyze' button is highlighted, and a menu is open showing the path: 'Export' -> 'Align / tree' -> 'from Export field' -> 'using MAFFT web site' -> 'Copy Export Field to clipboard and open MAFFT web site'. A sub-menu for 'using MAFFT web site' provides instructions: '[1] paste into 'Input' field and click 'Submit'', '[2] click 'Phylogenetic tree'', '[3] click 'Go'', and '[4] click 'view tree on Phylo.io''. The right panel shows a 'Sequence analysis' window with search results for 'DNA_Anna.txt_(PCR)' and 'DNA_Boyfriend.txt_(PCR)', displaying their respective DNA sequences.

[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 it is described as a "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 "Multiple sequence alignment and NJ / UPGMA phylogeny" section with an "Input:" field. A right-click 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, and Random Agent Spoofer. The "Check Spelling Languages" option is checked. The "Block Element" option has a red lock icon. The "Random Agent Spoofer" option has a blue globe icon. The "Allow unusual symbols (Selenocysteine "U", Inosine "i", non-alphabetical)" option is unchecked. The background shows a file explorer window with a list of files and a terminal window with a sequence alignment.

Back to beginning

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

The screenshot shows a computer interface with two main windows. On the left is a file manager window titled "Opened & processed" with a list of files:

- 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)

At the bottom of the file manager, there are buttons for "Quick Load / Run", "Analyze", and "? 7 files opened".

On the right is a web browser window titled "MAFFT alignment and NJ / ...". The address bar shows "https://mafft.cbrc.jp/alignm...". The page title is "MAFFT version 7" and the subtitle is "Multiple alignment program for amino acid or nucleotide sequences". The logo for CBRC and AIST is visible.

The main content area of the browser shows "Multiple sequence alignment and NJ / UPGMA phylogeny". Under the "Input:" section, there is a text area containing the following DNA sequences:

```
>DNA_Anna.txt_(PCR)
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT

>DNA_Boyfriend.txt_(PCR)
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT

>DNA_Boyfriends_partner.txt
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT

>DNA_Local_Control_12.txt_(
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAAGTAGTAATTAGATCTGAAAATTT
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAATGCTAAAATCAT

>DNA_Local_Control_22.txt_(
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCACAAATTCCTGGACAAATGCTAGAACCAT
AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCACAAATTCCTGGACAAATGCTAGAACCAT
```

Below the text area, there is a "Browse..." button and the text "No file selected.". There are also checkboxes for "Use structural alignment(s)" and "Allow unusual symbols (Selenocysteine 'U', Inosine 'i', non-alphabetical characters, etc.)" and a "Help" link.

At the bottom left of the image, there is a blue link that says "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 screenshot displays the MAFFT version 7 web interface. The browser address bar shows the URL <https://mafft.cbrc.jp/align/>. The page title is "MAFFT version 7" and the subtitle is "Multiple alignment program for amino acid or nucleotide sequences". The interface includes a navigation menu with links for "Download version" (Mac OS X, Windows, Linux, Source), "Online version" (Alignment, mafft --add, Merge, Phylogeny, Rough tree), "Merits / limitations", "Algorithms", "Tips", "Benchmarks", and "Feedback". The "Direction of nucleotide sequences" section has three radio button options: "Same as input" (selected), "Adjust direction according to the first sequence (accurate enough for most cases)", and "Adjust direction according to the first sequence (only for highly divergent data; extremely slow)". The "Output order" section has two radio button options: "Same as input" and "Aligned" (selected). The "Notify when finished" section has an "Email address:" input field. The "Submit" and "Reset" buttons are visible at the bottom of the main form. The "Advanced settings" section is partially visible, showing the "Strategy" section with "Auto (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depends on data size) Updated" selected. On the left, a file manager window titled "Opened & processed" lists several DNA files, including "DNA_Local_Control_3.txt". A text editor window shows the first 5 characters of a file: "AGCCCGATACG ACCATAATAGT GACCCGGGAAA ATGACACTTTA GGACCCAGAAA". A "Back to beginning" link is located at the bottom left of the image.

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 alignment... x +

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

Most Visited | News | UWRF | Wikipedia | Case It | YouTube | Prostate

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 agcccgatacgcataagatgaggtagtaattagatctgcc----
DNA_Boyfriend.t agcccgatacgcataagatgaggtagtaattagatctgcc----
DNA_Boyfriends_ agcccgatacgcataagatgaggtagtaattagatctgcc----
DNA_Local_Contr agcccgatacgcataagatgaggtagtaattagatctgac----
DNA_Local_Contr agcccgatacgcataagatgaggtagtaattagatctgccgaag
DNA_Local_Contr agcccgatacgcataagatgaggtagtaattagatctgccgagg
*****
DNA_Anna.txt_(P aatttcacagacaatgctaaaaatcataatagtagcagctgaatg
DNA_Boyfriend.t aatttcacagacaatgctaaaaatcataatagtagcagctgaatg
DNA_Boyfriends_ aatttcacagacaatgctaaaaatcataatagtagcagctgaatg
```

Back to beginning

Be careful if there are

Transferring data from mafft.cbrc.jp...

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 alignment tool interface:

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

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

Open all plots

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):

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

AGCCCGATACG
ACCATAAATAGT
GACCGGGGAAA
ATGACACTTTA
GGACCCAGAAA

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

...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

Filename: primers
FP: 5' -> AGCCC
RP: 5' -> TACAC

'DNA_Local_C
The first 5 cha
This file contai

Original order
1

AGCCCGATACG
ACCATAATAGT
GACCGGGGAAA
ATGACACTTTA
GGACCCAGAAA

Genbank | Lab Ben

Multiple sequence alignment... x +

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

Most Visited | News | UWRF | Wikipedia | Case It | YouTube | Prostate

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

[Open all plots](#)

Phylo.io runs on any modern browser.

[View tree on Phylo.io](#)

[Refine dataset on tree](#) **Alpha testing, 2016/Aug**

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

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

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

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

GGTA
ACAG
TACA
TTAA
TACA
ACCA
AGAT
AGAA
AGAA
CTGG
AATT
TAAT
AGAA

GGTA
ACAG
TACA
TTAA
TACA
ACCA
AGAA
AGAA
ACGA
AGAA
CAGG

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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.

The image shows a screenshot of the Phylo.io web interface. The browser address bar displays the URL: `https://mafft.cbrc.jp/alignment/server/spool/_phyloio.17112503531091.html`. The interface includes a sidebar on the left with a list of files under "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)

Below this list are buttons for "Quick Load / Run", "Analyze", and "? 7 files open". The main interface features a "Phylo.io" header with "Version: 1.0.k" and "View" / "Compare" buttons. A "Tree:" section contains a partial Newick tree format:

```
(  
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
```

A "Render" button is located below the tree. The central area displays a phylogenetic tree with six tips labeled: 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, and 2_DNA_Boyfriend_txt_PCR. A "Zoom:" control with directional arrows is positioned above the tree. A search box is in the top right. The bottom of the interface shows a scale bar with the value "0.16" and a "Settings" button. The footer includes "© Dessimoz Lab".

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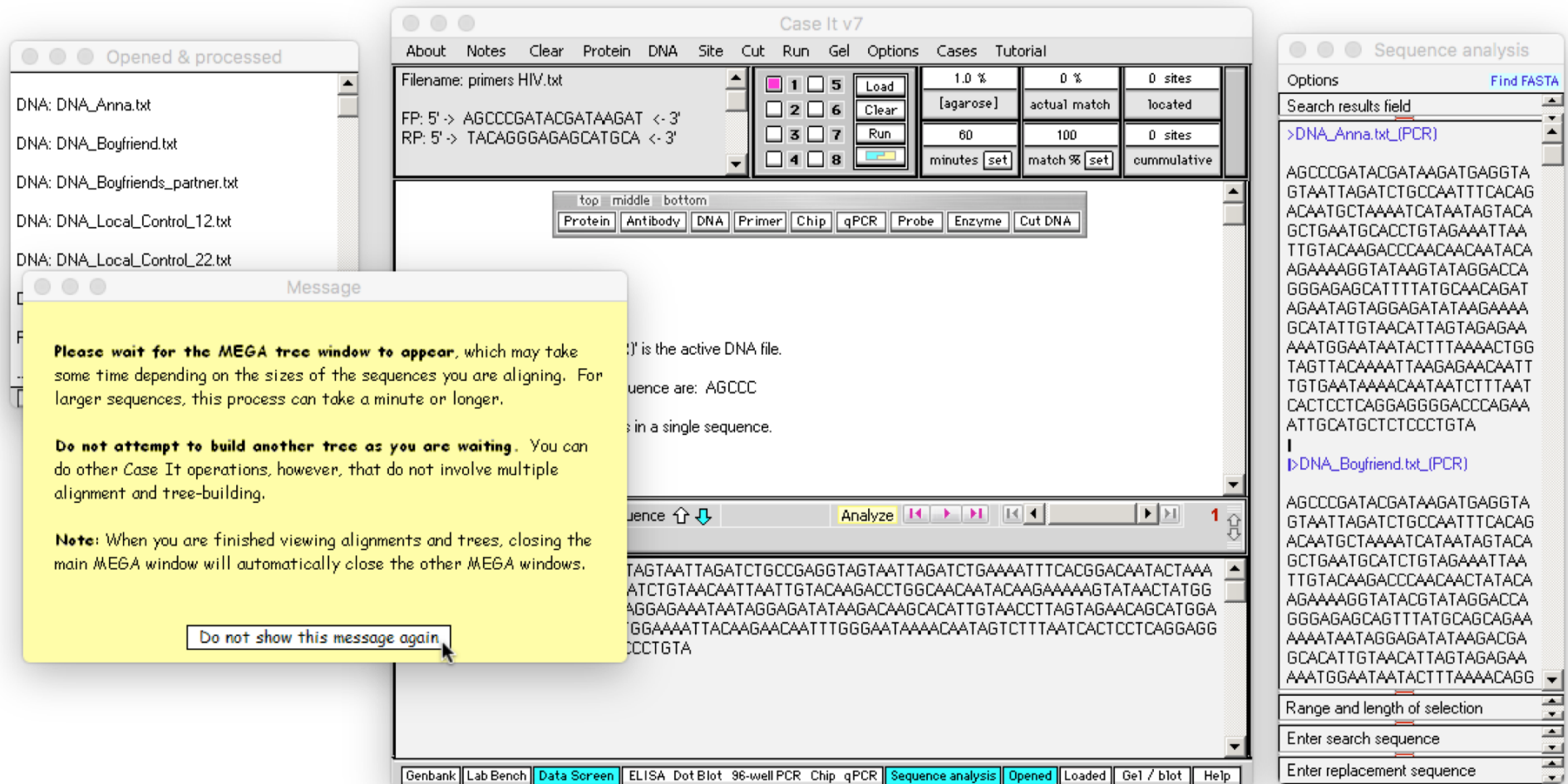
The third option for multiple alignment and tree-building is to have Case It open and control MEGA5 software. If default values for this software are used, then Case It will open MEGA5 and build a tree with one click (assuming that the MEGA5 executable is in the MEGA folder of Case It – see “Installation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

The screenshot displays the Case It v7 software interface. On the left, a list of files is shown under 'Opened & processed', including DNA files and primers. The main window shows the 'primers HIV.txt' file with forward and reverse primer sequences. A menu is open over the 'Analyze' button, showing the path: **Analyze** > **Align / tree** > **from Export field** > **using MEGA software** > **show alignment and tree**. The 'show alignment and tree' option is highlighted. Below this menu, a warning message states: 'MEGA5.22 must be installed - CLICK HERE for instructions. It may take awhile for the above commands to work, depending on sequence size.'

On the right, the 'Sequence analysis' window shows search results for '>DNA_Anna.txt(PCR)' and '>DNA_Boyfriend.txt(PCR)', displaying DNA sequence alignments.

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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).

The screenshot displays the MEGA 5.05 software interface. A 'Confirm' dialog box is centered, asking if the user wants to download and install an update. The 'Ignore' button is highlighted. In the background, the main MEGA 5.05 window is visible, showing a menu bar with options like 'Align', 'Data', 'Models', 'Distance', 'Diversity', 'Phylogeny', 'User Tree', 'Ancestors', 'Selection', 'Rates', and 'Clocks'. A 'Sequence analysis' window is open on the right, displaying sequence data for two files: '>DNA_Anna.txL(PCR)' and '>DNA_Boyfriend.txL(PCR)'. The 'Sequence analysis' window has a 'Find FASTA' button and a search results field. The main MEGA 5.05 window also shows a 'Data Screen' tab and a 'Sequence analysis' tab. The status bar at the bottom indicates 'GA release #5110426' and 'Sequence analysis' is the active window.

MEGA 5.05

Confirm

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.

Yes No Ignore

MEGA 5

Sequence analysis

Options Find FASTA

Search results field

>DNA_Anna.txL(PCR)

AGCCCGATACGATAAGATGAGGTA
GTAATTAGATCTGCCAATTTACAG
ACAATGCTAAAAATCATAATAGTACA
GCTGAATGCACCTGTAGAAAATTA
TTGTACAAGACCCCAACAATACA
AGAAAAGGTATAAGTATAGGACCA
GGGAGAGCATTTTATGCAACAGAT
AGAATAGTAGGAGATATAAGAAAA
GCATATTGTAACATTAGTAGAGAA
AAATGGAAATAACTTTAAAACCTGG
TAGTTACAAAATTAAGAGAACCAAT
TGTGAATAAAAAATAATCTTTAAT
CACTCCTCAGGAGGGACCCAGAA
ATTGCATGCTCTCCCTGTA

>DNA_Boyfriend.txL(PCR)

AGCCCGATACGATAAGATGAGGTA
GTAATTAGATCTGCCAATTTACAG
ACAATGCTAAAAATCATAATAGTACA
GCTGAATGCATCTGTAGAAAATTA
TTGTACAAGACCCCAACTATACA
AGAAAAGGTATACGTATAGGACCA
GGGAGAGCAGTTTATGCAGCAGAA
AAAAAATAGGAGATATAAGACGA
GCACATTGTAACATTAGTAGAGAA
AAATGGAAATAACTTTAAAACAGG

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

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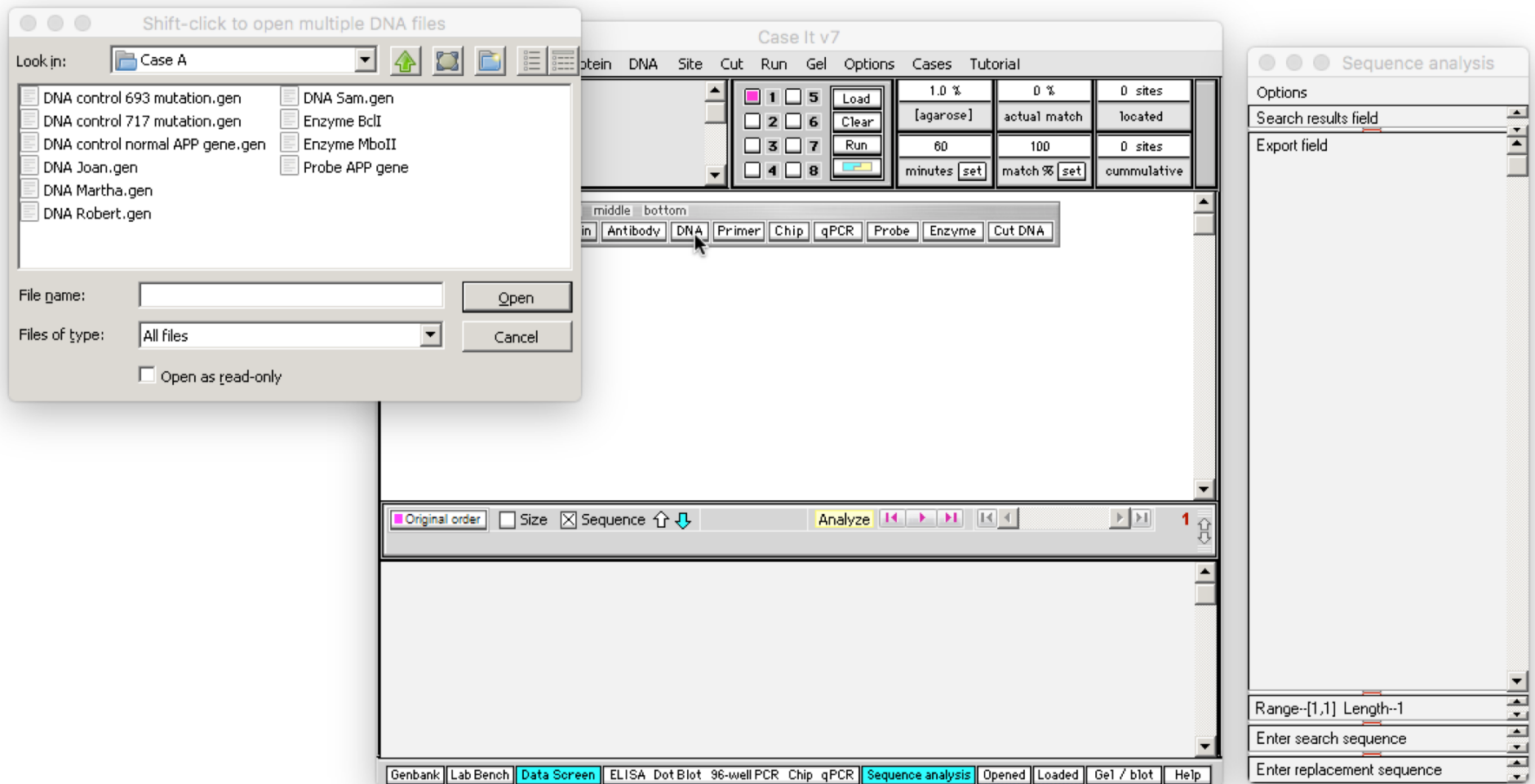
The multiple alignment and tree now appear. The advantage of using MEGA5 over the bioinformatics sites has do do with the many manipulations of the alignment and tree that are possible using MEGA5.

The screenshot displays the MEGA5 software interface, which is used for molecular evolutionary genetics analysis. It features several windows:

- M5: Alignment Explorer (out.fasta):** This window 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 PThe alignment is color-coded by nucleotide (A, C, G, T) and includes a gap character (-). A scale bar at the bottom indicates a distance of 0.01 substitutions per site (SBL = 0.26514000).
- M5: Tree Explorer (tree.nwk):** This window displays a phylogenetic tree based on the alignment. The tree shows the relationships between the sequences, with a scale bar of 0.01. The sequences are labeled as:
 - DNA Anna.txt PCR
 - DNA Boyfriend.txt PCR
 - DNA Boyfriends partner.txt
 - DNA Local Control 22.txt
 - DNA Local Control 12.txt
 - DNA Local Control 3.txt P
- Sequence analysis:** This window shows the sequence analysis results, including the FASTA format of the sequences.
- Opened & processed:** This window lists the files loaded into the software, including DNA sequences and primers.

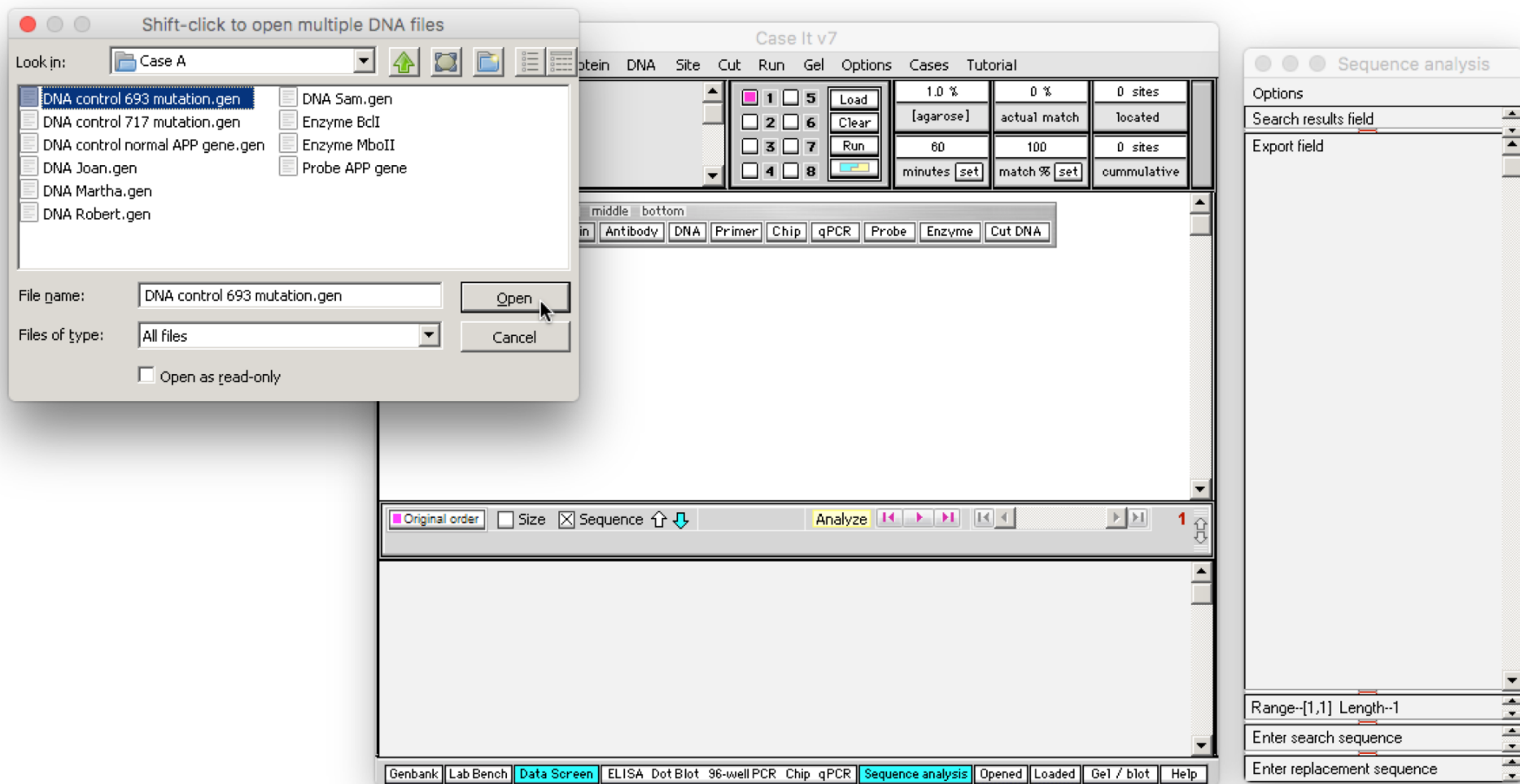
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Case It can also be used as a front end for BLASTING DNA and protein sequences. We'll use one scenario from the Alzheimer's case as an example. Click the **DNA** button on the silver button bar, and navigate to **Cases -> Genetic disease cases -> Alzheimer's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

The screenshot displays the Case It v7 software interface. On the left, the 'Opened & processed' window shows the file 'DNA: DNA_control_693_mutation.gen'. The main window, titled 'Case It v7', has a menu bar (About, Notes, Clear, Protein, DNA, Site, Cut, Run, Gel, Options, Cases, Tutorial) and a control panel with buttons for 'Load', 'Clear', 'Run', and a progress indicator. A table of parameters is visible:

| | | |
|------------------------------|------------------------------|-------------|
| 1.0 % | 0 % | 0 sites |
| [agarose] | actual match | located |
| 60 | 100 | 0 sites |
| minutes <input type="text"/> | match % <input type="text"/> | cummulative |

Below the control panel are buttons for 'top', 'middle', 'bottom' and 'Protein', 'Antibody', 'DNA', 'Primer', 'Chip', 'qPCR', 'Probe', 'Enzyme', '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, a sequence list shows 'Original order', 'Size', and 'Sequence' columns. The 'Sequence' checkbox is checked, and a blue arrow points down. The sequence text is displayed below:

```

tcagaagatcaatgctgcccggttggcactgctcctgctggccgctggaaggctggggcgtggaggtagccactgatggaatgctggcctgctggtgaaccca
gattgccatgtctgtggcagactgaacatgcacatgaatgcccagaatgggaagtgggalltcagatccatcagggaccacaaacctgcaatgataccaaggaaggc
ctgcagtagtccaagaagctaccctgaactgcagatcaccatgtggaagaaccaaccagtgaccatccagaactggtgcaagcggggccgcaagcagt
gcaagaccatccccactttgtgattccctaccgctgcttagttgtagttgtaagtagtgcctctctgctgacaagtgcaaatcttacaccaggagaggatggatg
ttcgaaactcatctcactggcacaccgctgcgaagagacatgcagtgagaagagtagcaacttgcagactaccgcatgttgcctgcgggaaltgacaagltcc
gagggtagagttgtgtgcccactggctgaagaaagtgacaatgtggallctgctgatgcccggaggaggatgactcggatgctgtggggcggagcagacagac
tatgcagatgggagtgaagacaaagttagaagtagcagagggaagaagtgctgaggtggaagaagaagaagccgatgatcagaggacgatgaggatggt
gatgagtagaggaagaggctgaggaaccctacgaagaagccacagagagaaccaccagcattgccaccaccaccaccaccaccacagagctgtggaagag
gtggtcgagagggtgctctgaacaagccgagaccggggccgtgccgagcaatgatctcccgtgtactttgatgtgactgaaggaagtgccccattctttaccggc
ggatgtggcggcaaccggaacaaccggaacaacttgacacagaagagtagctgcatggcccgtgtggtggcagccattcctacaacagcagccagtagccctgatg

```

On the right, the 'Sequence analysis' window shows options for 'Search results field' and 'Export field'. At the bottom of the main window, a toolbar includes 'Analyze' and navigation buttons. The bottom status bar shows 'Data Screen' selected among other options like 'Genbank', 'Lab Bench', 'ELISA', 'Dot Blot', '96-well PCR', 'Chip', 'qPCR', 'Sequence analysis', 'Opened', 'Loaded', 'Gel / blot', and 'Help'.

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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 to 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 (About, Notes, Clear, Protein, DNA, Site, Cut, Run, Gel, Options, Cases, Tutorial) and a control panel with buttons for "Load", "Clear", "Run", and "minutes". A table of parameters is visible:

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

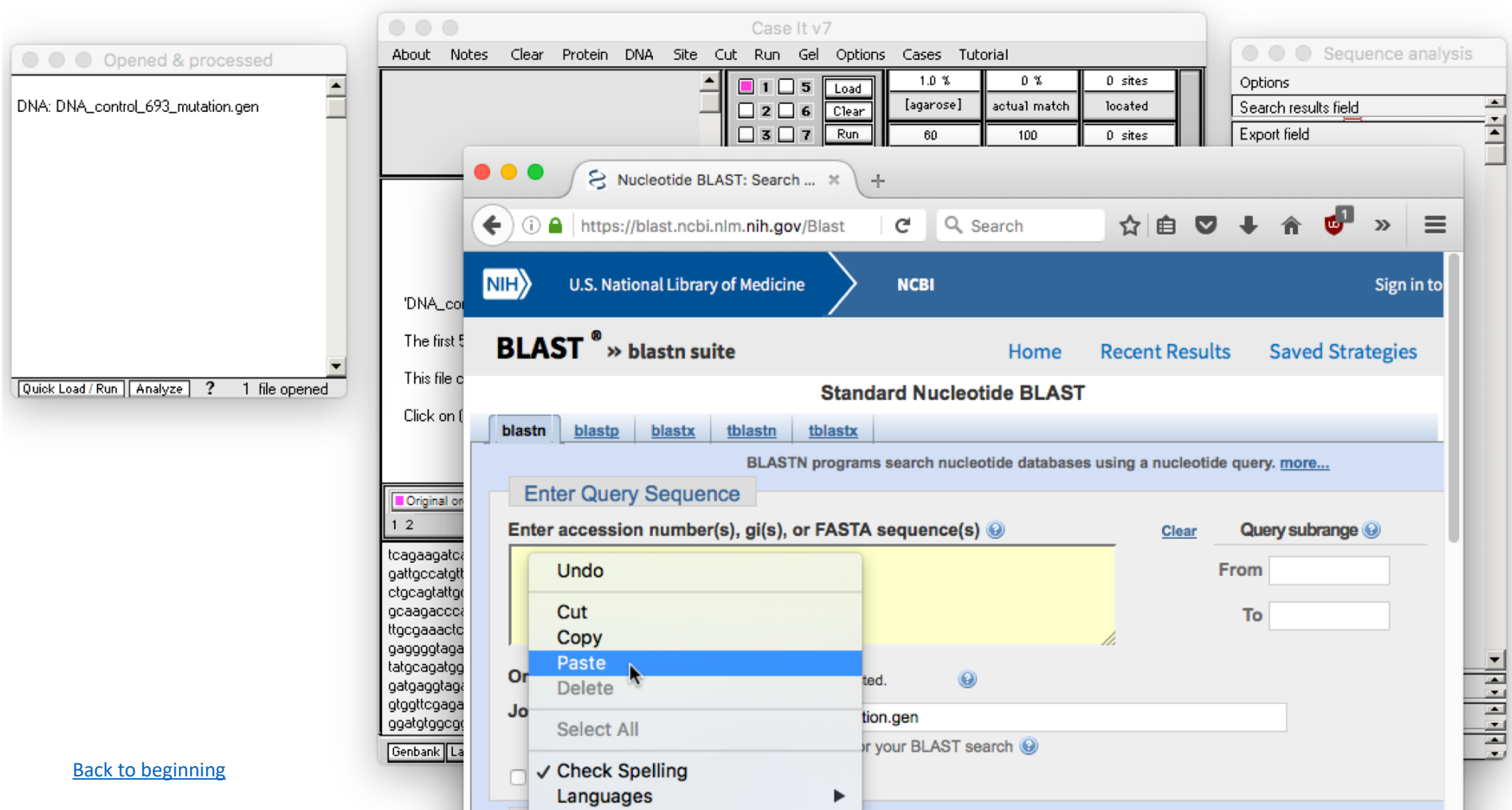
Below the control panel are buttons for "top", "middle", "bottom", "Protein", "Antibody", "DNA", "Primer", "Chip", "qPCR", "Probe", "Enzyme", and "Cut DNA". The main text area contains instructions: "'DNA_control_693_mutation.gen' is the active DNA file. The first 5 characters of the DNA sequence are: 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 is a sequence viewer with "Original order", "Size", and "Sequence" checkboxes, and navigation buttons. A DNA sequence is displayed, with a portion highlighted and a context menu open over it. The menu options are:

- 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, there is a toolbar with buttons for "Genbank", "Lab Bench", "Data Screen", "ELISA", "Dot Blot", "96-well PCR", "Chip", "qPCR", "Sequence analysis", "Opened", "Loaded", "Gel / blot", and "Help". A text input field "Enter replacement sequence" is also present.

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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.



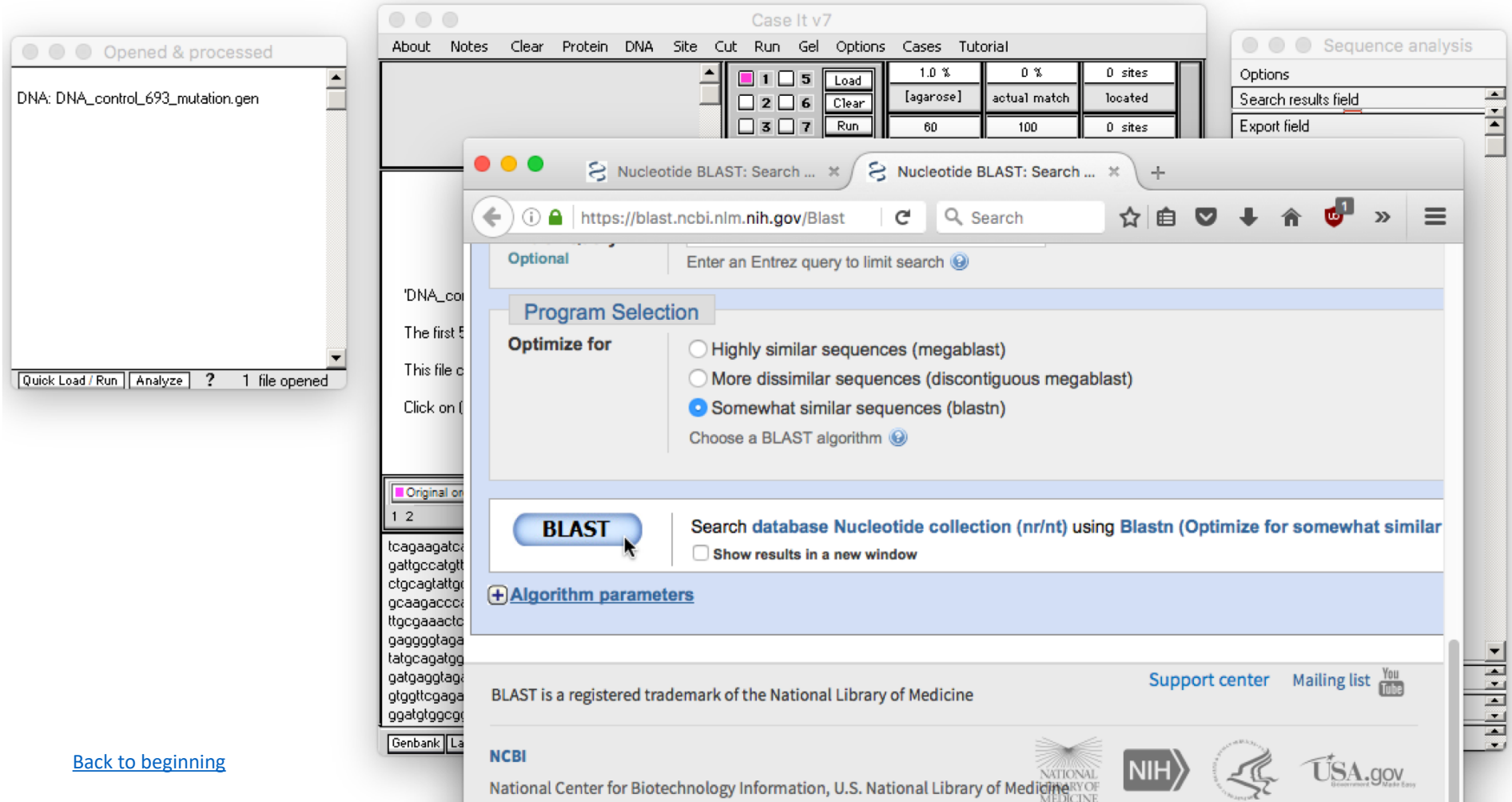
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The DNA sequence copied from Case It is not in the Query Sequence field of the NCBI site.

The screenshot displays a multi-windowed desktop environment. On the left, a window titled "Opened & processed" shows the text "DNA: DNA_control_693_mutation.gen". In the center, the "Case It v7" application is open, featuring a menu bar (About, Notes, Clear, Protein, DNA, Site, Cut, Run, Gel, Options, Cases, Tutorial) and a control panel with buttons for "Load", "Clear", and "Run", along with numerical input fields (1-7) and percentage settings (1.0%, 0%, 80%). On the right, a "Sequence analysis" window shows options for "Search results field" and "Export field". The foreground is dominated by a web browser window at "https://blast.ncbi.nlm.nih.gov/Blast". The browser shows the NCBI logo and "BLAST® >> blastn suite" header. The main content area is titled "Standard Nucleotide BLAST" and includes tabs for "blastn", "blastp", "blastx", "tblastn", and "tblastx". The "blastn" tab is active, showing the "Enter Query Sequence" section. This section contains a text input field with the following text: ">DNA_control_693_mutation.gen" followed by a sequence of nucleotides: "gattccctaccgctgctttagttggtgagtttgaagtgatgcccttctcgttctctgacaagtccaattcttacacc". The sequence is partially highlighted in red. Below the input field, there is a "Job Title" field containing "DNA_control_693_mutation.gen" and a checkbox for "Align two or more sequences".

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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.

The image shows a BLAST search interface. On the left, a file upload window titled "Opened & processed" shows the file "DNA: DNA_control_693_mutation.gen". Below it are buttons for "Quick Load / Run", "Analyze", and "?", and a status "1 file opened".

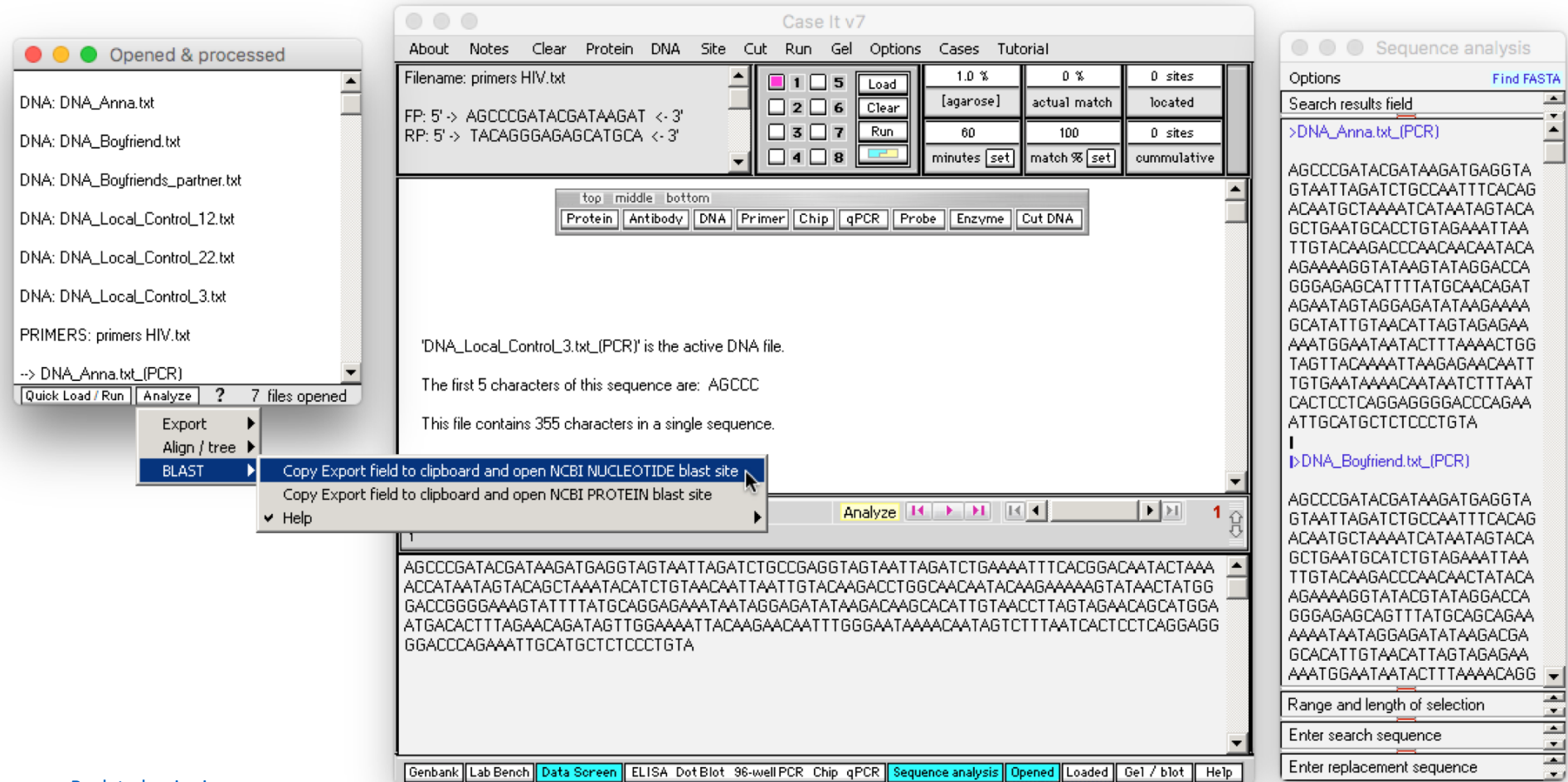
The main browser window is titled "Nucleotide BLAST: Search ..." and "NCBI Blast:DNA_control_69...". The address bar shows "https://blast.ncbi.nlm.nih.gov/Blast". The search results are displayed in a table under the heading "Sequences producing significant alignments:". The table has columns for "Description", "Max score", "Total score", "Query cover", "E value", and "Id".

| 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 |

Below the table, there is a "Back to beginning" link.

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).

The screenshot displays a multi-tasking desktop environment. On the left, a file manager 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. The selected file is DNA_Anna.txt (PCR). In the center, a text editor window shows the contents of "primers HIV.txt", displaying forward (FP) and reverse (RP) primer sequences: FP: 5' -> AGCCCGATACGATAAGATGAGGTAGTAATTAGATCTGCCAATTCACAGACAA and RP: 5' -> TACAGGGAGAGCATTTGCAATTAAGAGAAC. On the right, a web browser window is open to the NCBI Nucleotide BLAST search page (https://blast.ncbi.nlm.nih.gov). The page title is "BLAST >> blastn suite" and it shows the "Standard Nucleotide BLAST" interface. The "Enter Query Sequence" field is populated with the contents of the selected file, including headers like ">DNA Anna.txt_(PCR)", ">DNA Boyfriend.txt_(PCR)", and ">DNA Boyfriends_partner.txt". The "Job Title" field at the bottom of the interface is set to "6 sequences (DNA_Anna.txt_(PCR))".

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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.

The image shows a multi-window environment. On the left, a window titled "Opened & processed" lists several DNA files: DNA_Anna.txt, DNA_Boyfriend.txt, DNA_Boysfriends_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 a question mark icon, with a status bar indicating "7 files opened".

In the middle, a window titled "About Notes Clear Pro" shows a file named "primers HIV.txt" with sequence information: "FP: 5' -> AGCCCGATACGATA" and "RP: 5' -> TACAGGGAGAGCAT". Below this is a text area containing the first 5 characters of the sequence and a note that the file contains 355 characters. At the bottom, there are sorting options for "Original order" and "Size", and a "Data Screen" button.

The main window is a web browser displaying the "NCBI Blast:6 sequences (D...)" page. The address bar shows "https://blast.ncbi.nlm.nih.gov". The page title is "BLAST Results". Navigation links include "Home", "Recent Results", and "Save". Action links include "Edit and Resubmit", "Save Search Strategies", "Formatting options", and "Download". The job title is "6 sequences (DNA_Anna.txt_(PCR))".

The "Results for:" section shows a dropdown menu with the following options:

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

The table below the dropdown lists the results:

| RID | Query ID | Description | Molecule type | Query Length |
|-------|-------------|-----------------------------|---------------|--------------|
| 1: cl | Query_95934 | DNA_Anna.txt_(PCR) | | 337bp |
| 2: cl | Query_95935 | DNA_Boyfriend.txt_(PCR) | | 337bp |
| 3: cl | Query_95936 | DNA_Boysfriends_partner.txt | | 337bp |
| 4: cl | Query_95937 | DNA_Local_Control_12.txt_((| | 358bp |
| 5: cl | Query_95938 | DNA_Local_Control_22.txt_((| | 337bp |
| 6: cl | Query_95939 | DNA_Local_Control_3.txt_(P | | 355bp |

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#)

The "Graphic Summary" section is partially visible, showing a "Distribution of the top 100 Blast Hits on 100 subject sequence" and a "Color key for alignment scores" with categories: <40 (black), 40-50 (blue), 50-80 (green), and 80-200 (magenta). A horizontal bar chart shows the distribution of scores for the query, with a red bar at the bottom.

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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.

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 loaded, with forward and reverse primer sequences: FP: 5' -> AGCCCGATACGATAAGAT <- 3' and RP: 5' -> TACAGGGAGAGCATGCA <- 3'. The main display area shows the active DNA file and its first 5 characters (AGCCC). A context menu is open over the sequence, with "BLAST" selected, and a sub-menu showing options like "Copy Export field to clipboard and open NCBI NUCLEOTIDE blast site". On the right, a "Sequence analysis" window displays the full sequence and search results, including a "Find FASTA" button and search parameters like "Range-[1,355] Length-355".

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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.

The screenshot displays the Case It v7 software interface, which is used for DNA analysis. It consists of three main windows:

- Opened & processed:** A list of files including DNA sequences and primers. The active file is 'DNA_Anna.txt (PCR)'.
- Case It v7 (Main Window):** Shows the loaded file 'primers HIV.txt' with forward (FP) and reverse (RP) primer sequences. It includes a control panel with buttons for 'Load', 'Clear', and 'Run', and a table for analysis parameters:

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

 Below this, there are tabs for 'top', 'middle', and 'bottom', and a row of analysis options: Protein, Antibody, DNA, Primer, Chip, qPCR, Probe, Enzyme, and Cut DNA. The main text area indicates that 'DNA_Local_Control_3.txt (PCR)' is the active DNA file, with the first 5 characters being 'AGCCC'.
- Sequence analysis:** A window showing search results for 'DNA_Anna.txt (PCR)'. It displays a DNA sequence with a highlighted region. A context menu is open over the highlighted text, with the option 'Copy selected text to clipboard including FASTA definition line, and open NCBI blast site' selected.

At the bottom left, there is a link: [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. A 'DNA chip navi...' window is open, showing a grid of features. The main window displays a data table with columns 1 through 6 and rows A through H. A context menu is open over a selected feature, offering options to copy the probe sequence to the clipboard and open the NCBI nucleotide BLAST site, or to copy the SNP ID to the clipboard and open the SNP database in the default browser. The 'Sequence analysis' window is also visible on the right side of the interface.

| | 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 3392 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 |

Context menu 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
- Help

Buttons at the bottom: Protein, Antibody, DNA, Primer, Chip, qPCR, Probe, Enzyme, Cut DNA

Buttons at the bottom right: Genbank, Lab Bench, Data Screen, ELISA, Dot Blot, 96-well PCR, Chip, qPCR, Sequence analysis, Opened, Loaded, Gel / blot, Help

Buttons at the bottom left: Quick Load / Run, Analyze, ? 3 files opened

Buttons at the top: Load, Clear, Run

Options at the top right: 1.0 %, 0 %, 0 sites, [agarose], actual match, located, 60, 100, 0 sites, minutes [set], match % [set], cumulative

Buttons at the top: DNA, Site, Cut, Run, Gel, Options, Cases, Tutorial

Buttons at the top left: Opened & processed

Buttons at the top right: Sequence analysis

Buttons at the top: DNA chip navi...

Buttons at the top: Case It v7

Buttons at the top: Range and length of selection, Enter search sequence, Enter replacement sequence

Buttons at the top: This chip: Jonathan, Probe: gtoct

Buttons at the top: Select method, Load, Run, Options, Light, Labels, Load, Clear

Buttons at the top: SNP chip

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