

## Tutorial for using Case It to search DNA sequences

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[General sequence searching](#)

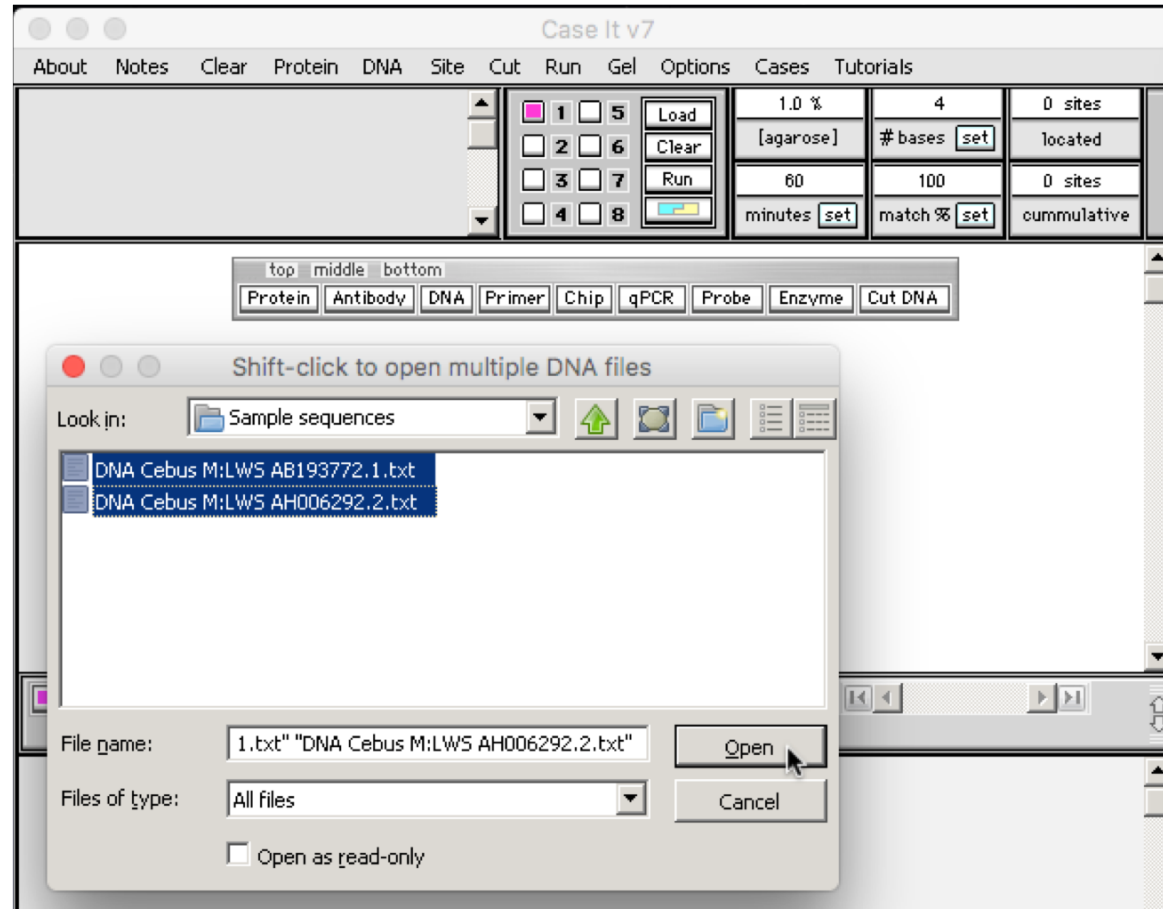
[Setting search parameters for the PCR procedure](#)

[Setting search parameters for Southern and dot blots](#)

[Obtaining sequences for research purposes](#)

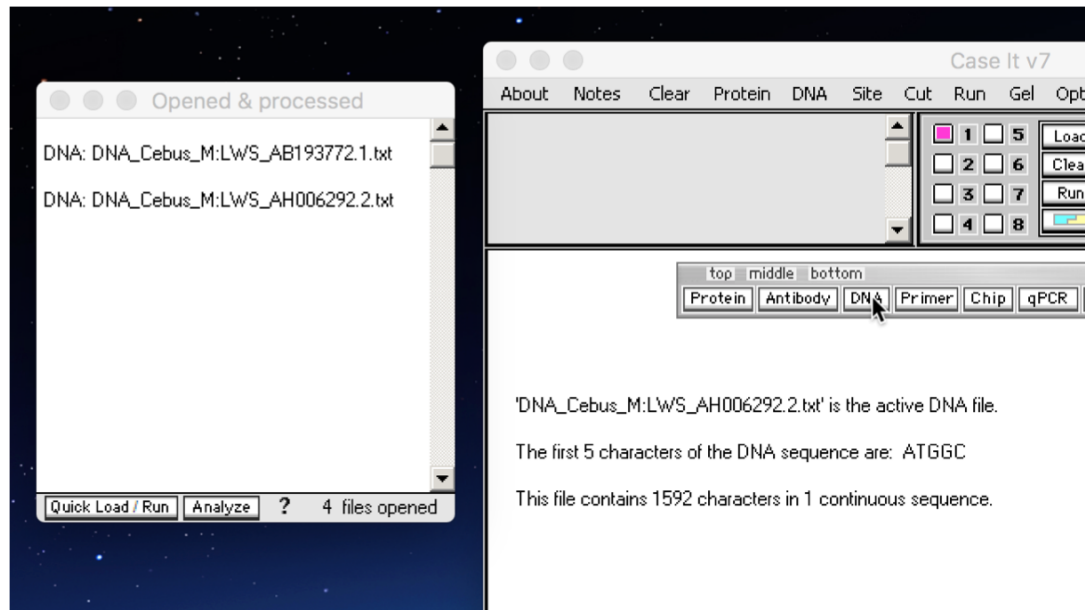
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The first part of this tutorial shows the basic procedure for using Case It v7 to search DNA sequences. Click the **DNA** button on the silver button bar, and double-click on these folders: **Case It V7 PC -> Cases -> Color Vision -> Sample Sequences**. Shift-click on the two sequences inside the **Sample Sequences** folder to highlight them, and then click the **Open** button:



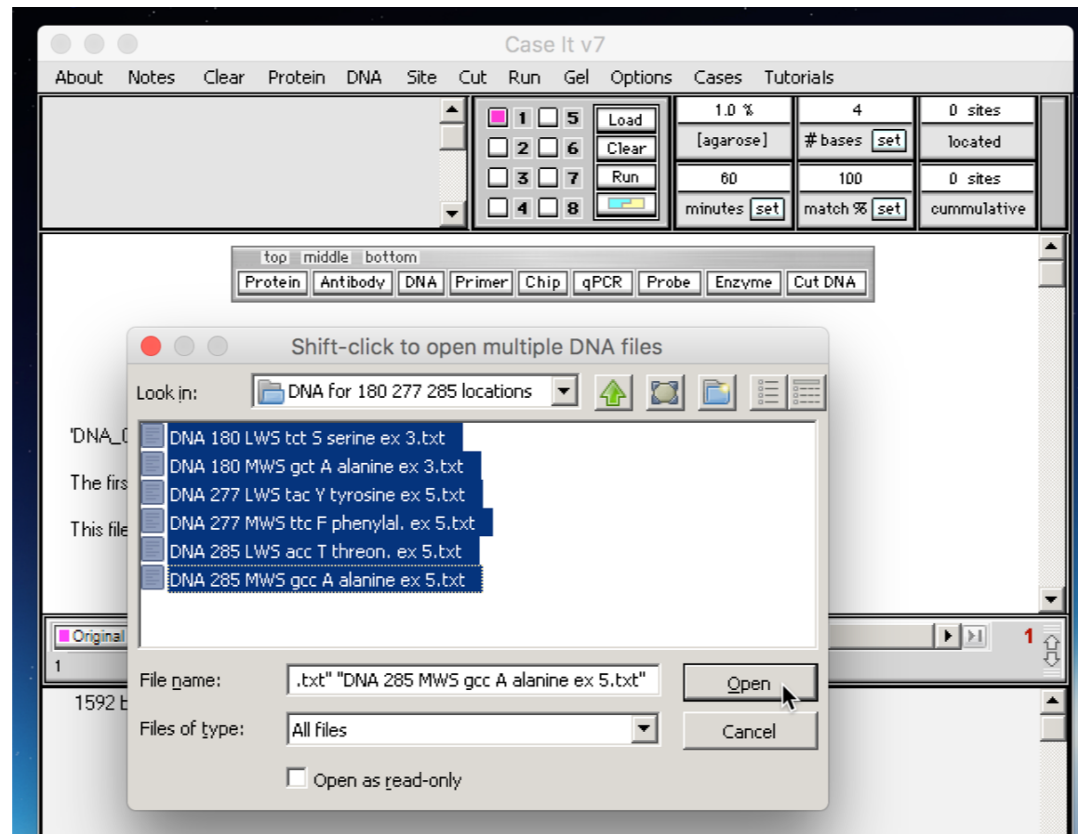
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The two open files appear in the **Opened & processed** window. Click the DNA button again...



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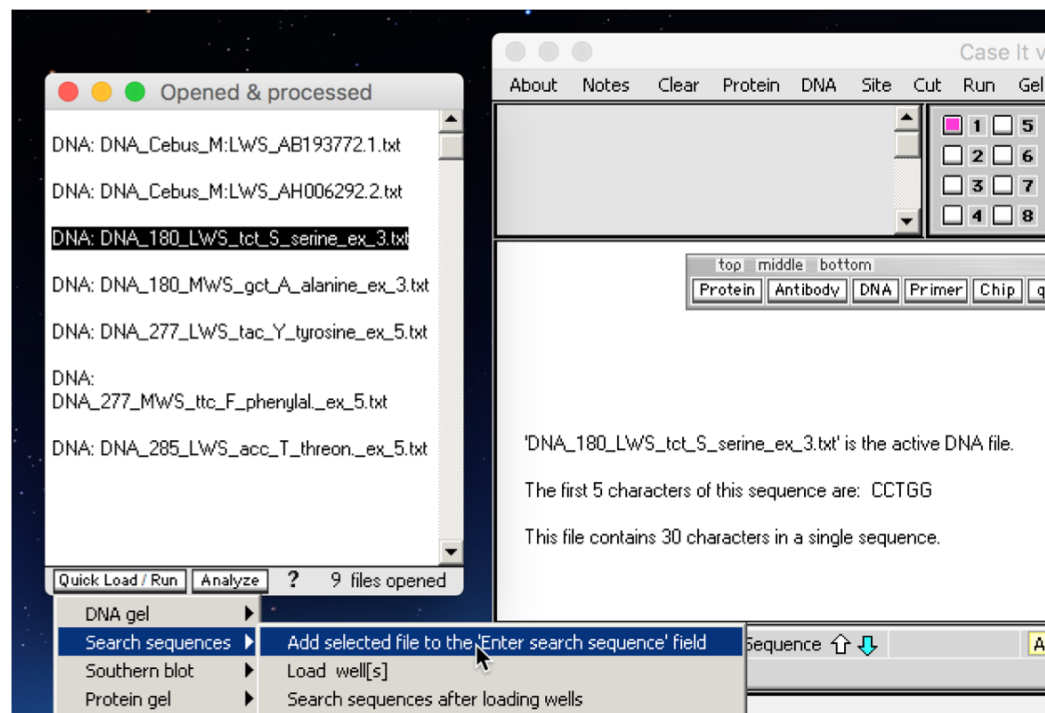
...and double-click through these folders: **Case It v7 PC -> Cases -> Color Vision -> DNA for 180 277 285 locations**. Shift-click to open all of the files in the **DNA for 180 277 285 locations** folder.



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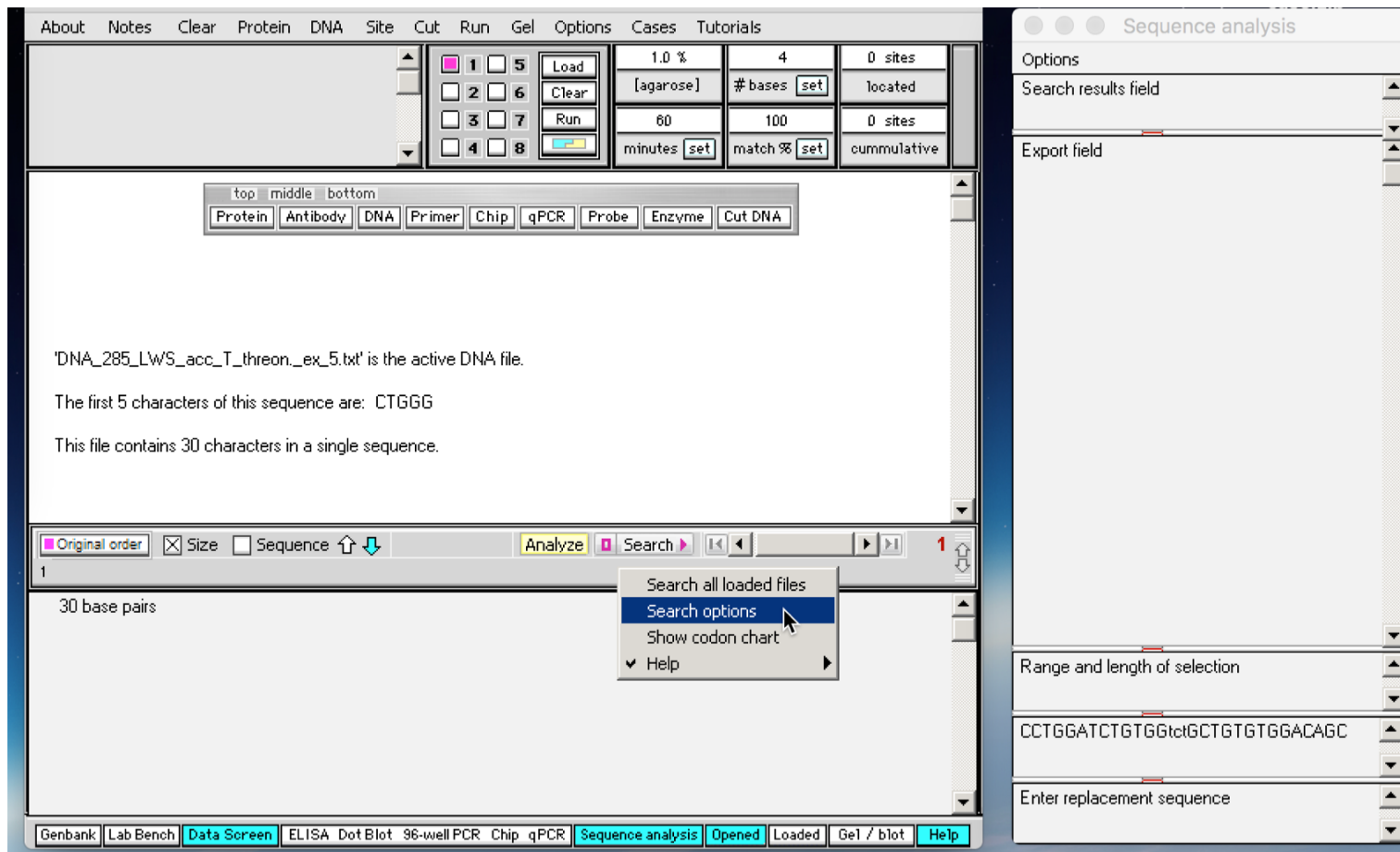
Click on the line **DNA: DNA\_180\_LWS\_tct\_S\_serine\_ex\_3.txt**, and then click the **Quick Load / Run** button and select **Search sequences -> Add selected file to the 'Enter search sequence' field**.

Note: The explanation of the filename is as follows: In Case It, DNA files must begin with the characters **DNA**. The number **180** refers to a location on Exon 3 (designated here as **ex\_3**) of the opsin gene, and the characters **tct** refer to the DNA codon associated with the amino acid **serine (S)**. One of these three characters would be at position 180 on exon 3 of the opsin gene. Color vision in primates and many other animals depend on which amino acid is associated with this and other positions on the opsin gene. For more information, visit the **Evo-Ed** site at <http://www.evo-ed.org> and click on **Monkey Opsins**.



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The 30-character sequence in this file appears in the second field from bottom of the **Sequence Analysis** window (lower right in the screen shot below). Click the **Search** button and select **Search options** (or click the purple button to the left of the Search button).



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Drag over the search sequence to determine the position of the three lowercase characters. As you do so, the length is updated in the Range field just above the search string field, and the highlighted characters appear there. Note that the three lowercase letters are in positions 14 through 16, so enter those numbers in the Search Options box, as shown on the next slide...

nce are: CTGGG  
a single sequence.

Starting and ending positions:  to

Restrict search results summary to this range

Capitalize  Boldface

Close Apply settings to search sequence →

Export field

Range-[1,16] Length--16  
CCTGGATCTGTGGtct

CCTGGATCTGTGGtctGCTGTGTGGACAGC

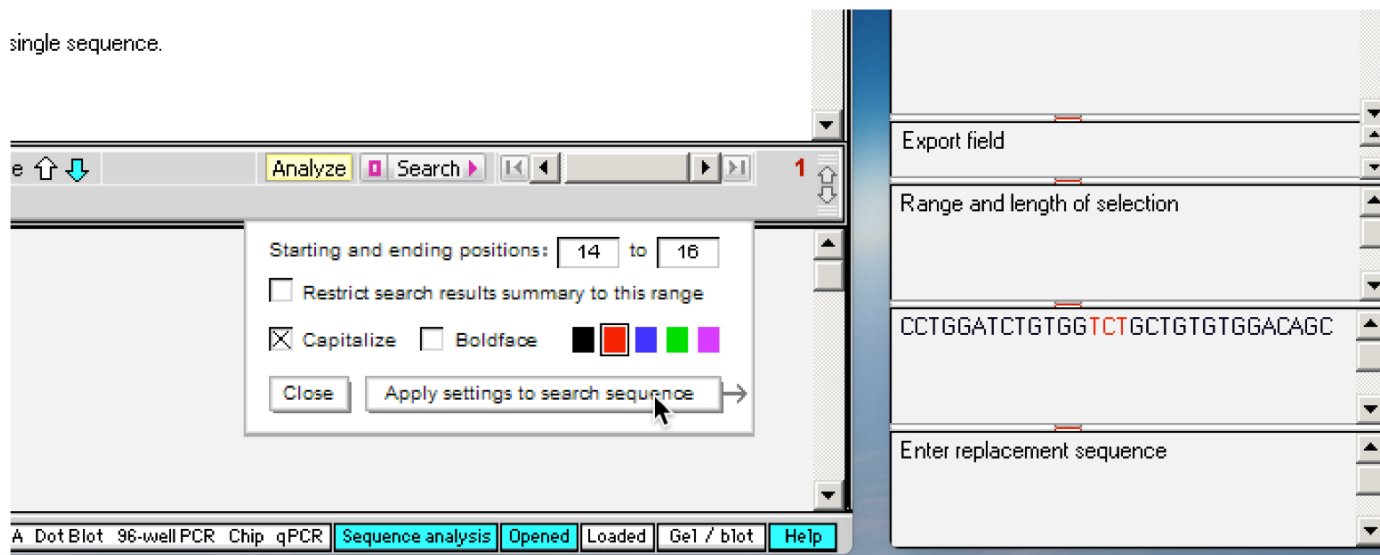
Enter replacement sequence

ISA Dot Blot 96-well PCR Chip qPCR Sequence analysis Opened Loaded Gel / blot Help

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After entering 14 and 16 as the starting and ending positions, click the **Apply settings to search sequence** button. The default is to highlight the starting and ending characters in red, and capitalize any lowercase letters, but these settings can be changed as shown below.

single sequence.

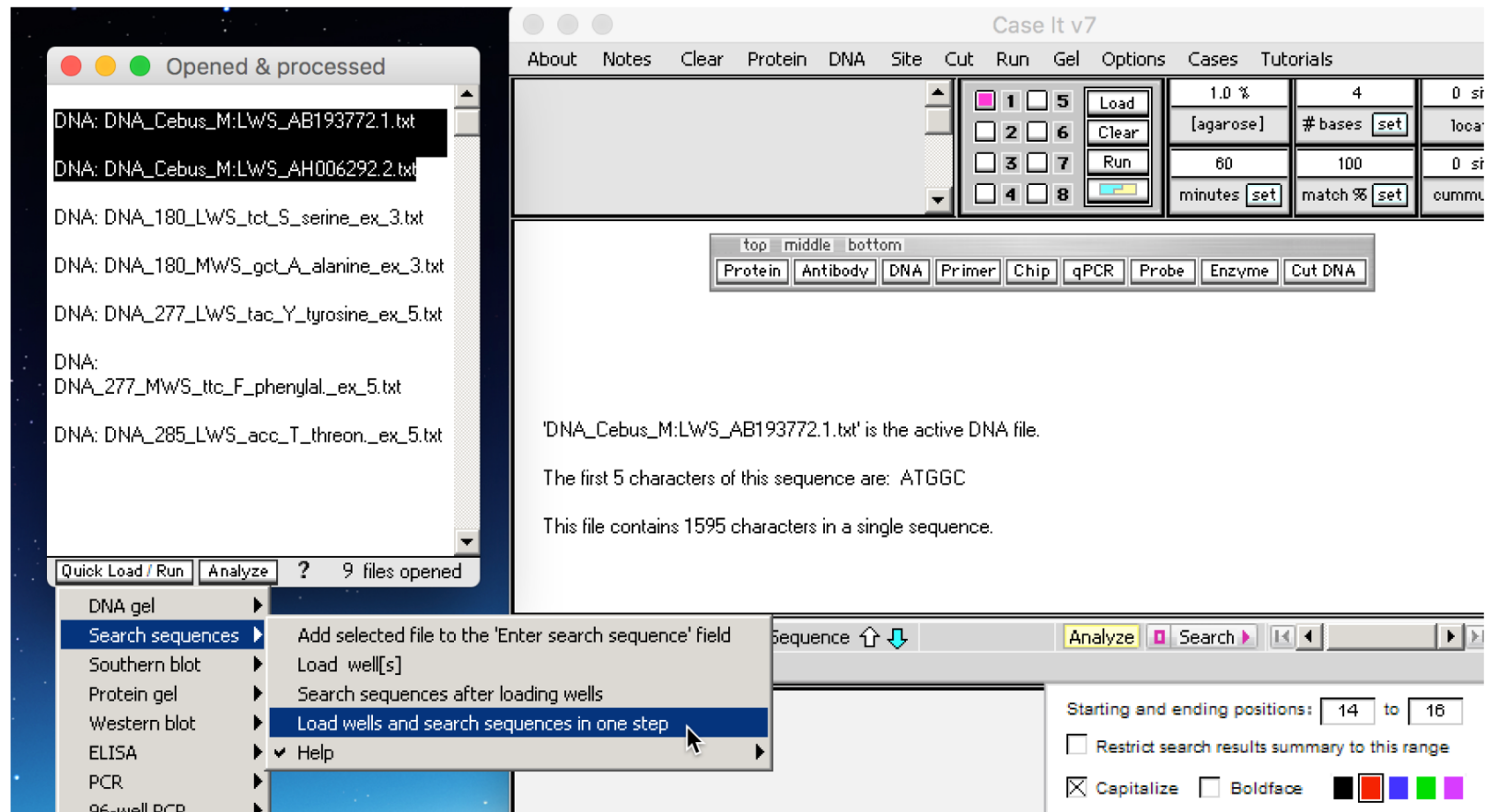


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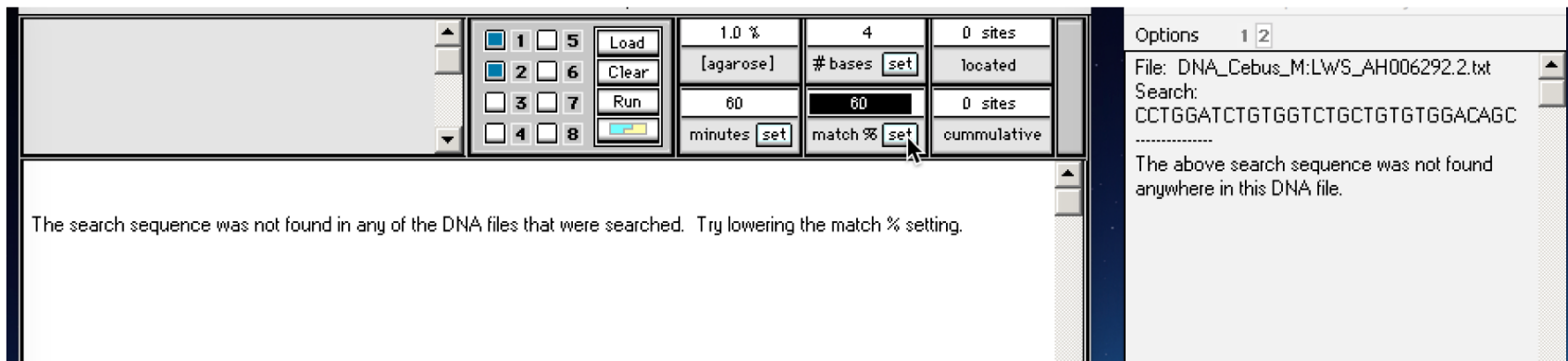
Highlight the two DNA sequences at the top of the **Opened & processed** window, then click the **Quick Load / Run** button of that window and select **Search sequences -> Load wells and search sequences in one step** as shown below. These sequences are from the Capuchin (genus Cebus), a New World monkey known for its tool use.

Note: For present purposes loading wells is not associated with running gels, but rather is a necessary step for the search procedure to work.

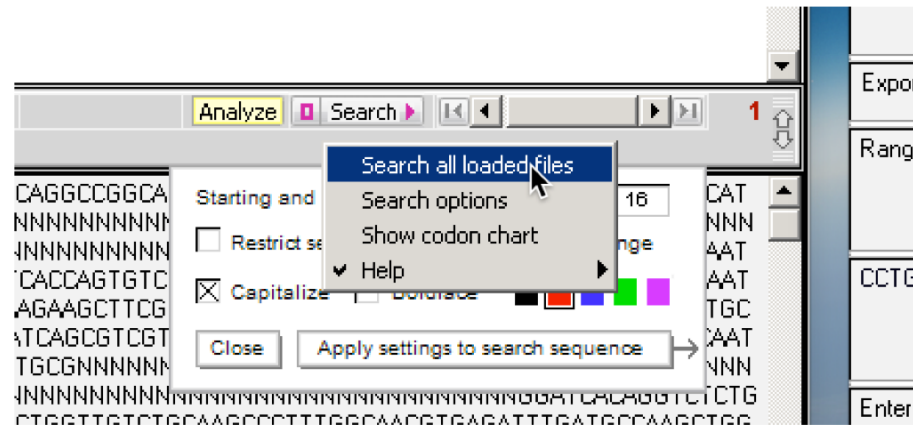


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A message appears that the 'search sequence was not found in any of the DNA files that were searched. Try lowering the match % setting.' Type **60** into the **match %** box and click the set button...



...and then click the **Search** button on the gray divider bar, and select **Search all loaded files** to research the files at this new match % setting.



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Results appear both in the white field of the **Data Screen** (as a summary of all results), and also in the **Search results** field of the **Sequence analysis** window (showing results for each file separately) Clicking the **1** or **2** at the top of the Sequence analysis window shows search results for the files loaded in either well **1** or well **2**. There was one 70% match for the first file searched (AB193772.1), and both 70% and 60% matches for the second file searched (AH006292.2). Note that the gray divider bar has been dragged down to show all results – the ‘drag handle’ for the divider bar is on its extreme right at the cursor location in the screen shot below.

The screenshot shows a software interface with a menu bar (About, Notes, Clear, Protein, DNA, Site, Cut, Run, Gel, Options, Cases, Tutorials) and a control panel with buttons for Load, Clear, Run, and Analyze. The main display area shows search results for three files:

```

DNA_Cebus_M:LWS_AB193772.1.txt
1: 725 - 754 [ 725 - 754 ] 70.00
CCTGGATCTGTGGTCTGCTGTGTGGACAGC
CTCCTGGATCTGGTCTGCTGTGTGGACGGC

DNA_Cebus_M:LWS_AH006292.2.txt
1: 725 - 754 [ 725 - 754 ] 70.00
CCTGGATCTGTGGTCTGCTGTGTGGACAGC
CTCCTGGATCTGGGCTGCTGTGTGGACAGC

DNA_Cebus_M:LWS_AH006292.2.txt
1: 727 - 756 [ 727 - 756 ] 60.00
CCTGGATCTGTGGTCTGCTGTGTGGACAGC
CCTGGATCTGGGCTGCTGTGTGGACAGCCC
  
```

The right-hand side panel, titled "Sequence analysis", shows search options and results for the selected file:

```

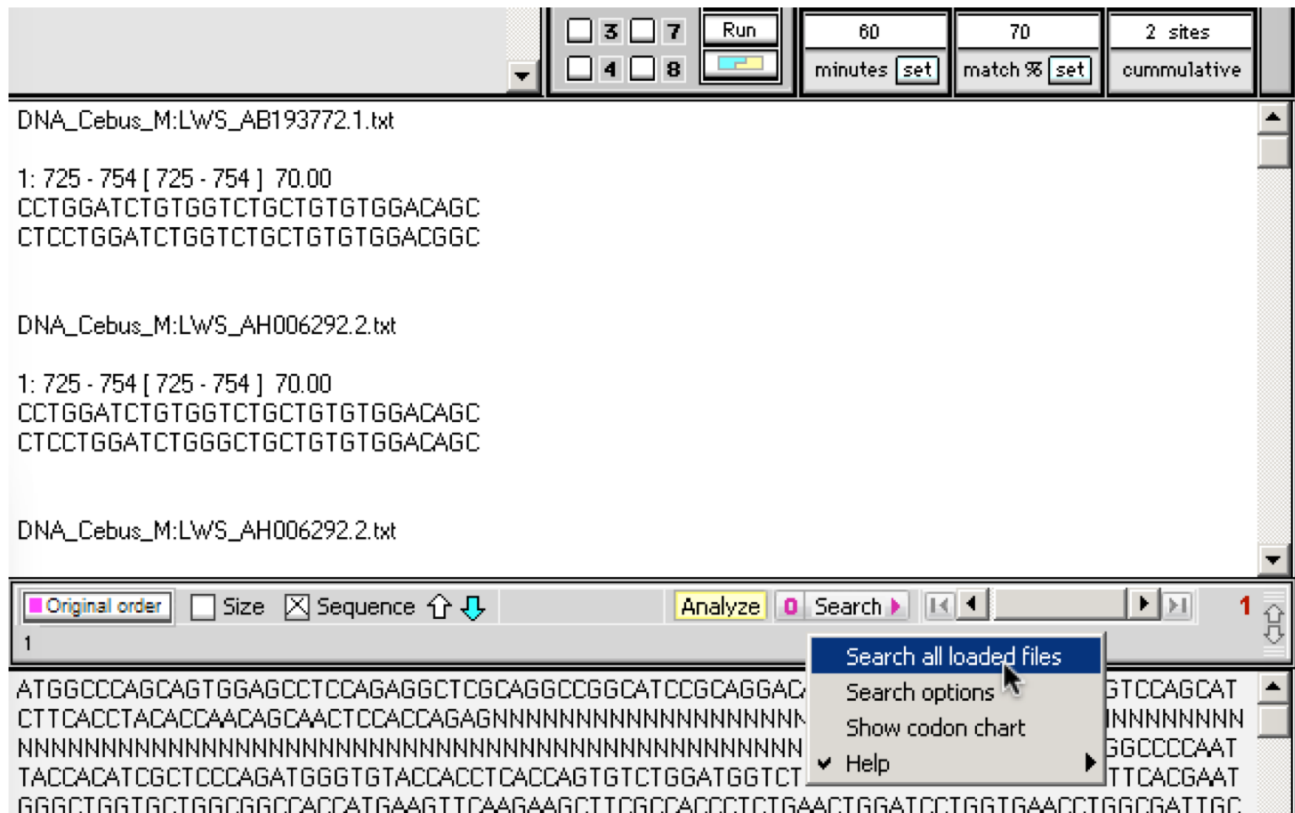
Options 1 2
File: DNA_Cebus_M:LWS_AH006292.2.txt
Search:
CCTGGATCTGTGGTCTGCTGTGTGGACAGC
-----
ORIGINAL ORDER
Fragment number, location of search sequence
within a particular fragment, [cumulative
location], and % match
-----
1: 725 - 754 [ 725 - 754 ] 70.00
1: 727 - 756 [ 727 - 756 ] 60.00
  
```

At the bottom of the right panel, the search sequence is highlighted in red: `CCTGGATCTGTGGTCTGCTGTGTGGACAGC`.

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Change the value in the match% box from 60 to 70, then click the **set** button. Use the **Search** button as shown below to select **Search all loaded files**. This command can also be given from the Quick Load / Run button of the Opened & processed window.

Note: The gray divider bar has been returned to its default position in the screenshot below. You can do this either by dragging the bar up by its drag handle, or by using the Analyze button and select Divider->Return gray divider bar to its default position (this step not shown here). You would now need to scroll to see all of the contents of the white field, but to activate the scrollbar you would need to first click in the white field, otherwise the scrollbar may pop up to its top position when released.



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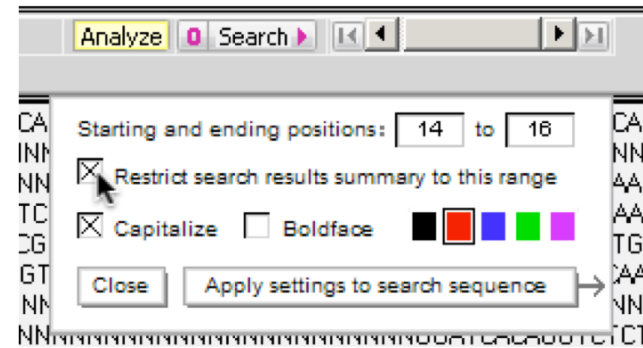
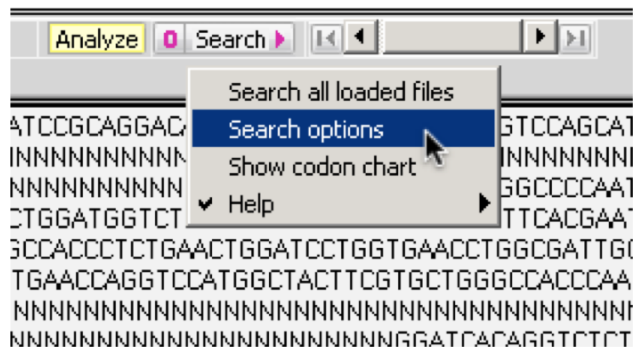
The files are searched again at the new setting of 70%, meaning that only hits with at least a 70% match are shown. Lines in the upper field of the Sequence analysis window represent hits, so if you click on one of these lines then the corresponding characters are highlighted in the file being searched, at the bottom of the Data Screen. In addition, those characters appear in the field at the bottom of the Sequence analysis window, so that you can compare them with the search string just above it. Note that in this case, the characters GCT were present in the file, whereas the characters in red just above it are TCT. What does this mean? Before explaining this, we'll use another Search Options feature to make it easier to see...

The screenshot displays a sequence analysis software interface. At the top, there is a control panel with buttons for 'Load', 'Clear', 'Run', and 'Exit'. Below these are input fields for '1.0 %' (set to 70), '# bases' (set to 4), and '1 sites' (set to 1). The main window is divided into two sections. The upper section shows search results for two files: 'DNA\_Cebus\_M:LWS\_AB193772.1.txt' and 'DNA\_Cebus\_M:LWS\_AH006292.2.txt'. Both files show a hit at position 1: 725 - 754 [ 725 - 754 ] 70.00. The lower section shows the original DNA sequence with the search string 'CCTGGATCTGTGGTCTGCTGTGTGGACAGC' highlighted in red. The search results are also displayed in the 'Options' panel on the right, which includes a 'Search' field with the same sequence and an 'Export field' button. Below the 'Export field' button, the search results are shown again, with the search string highlighted in red.

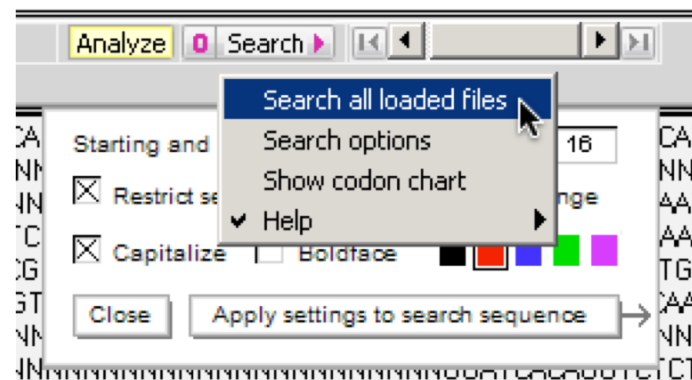
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Since the Search Options box had been closed in the preceding slide, open it again using the **Search** button (or by clicking the button with a purple O to the left of the Search button).

Click the checkbox next to **Restrict search results summary to this range**, so that an X appears in this checkbox.

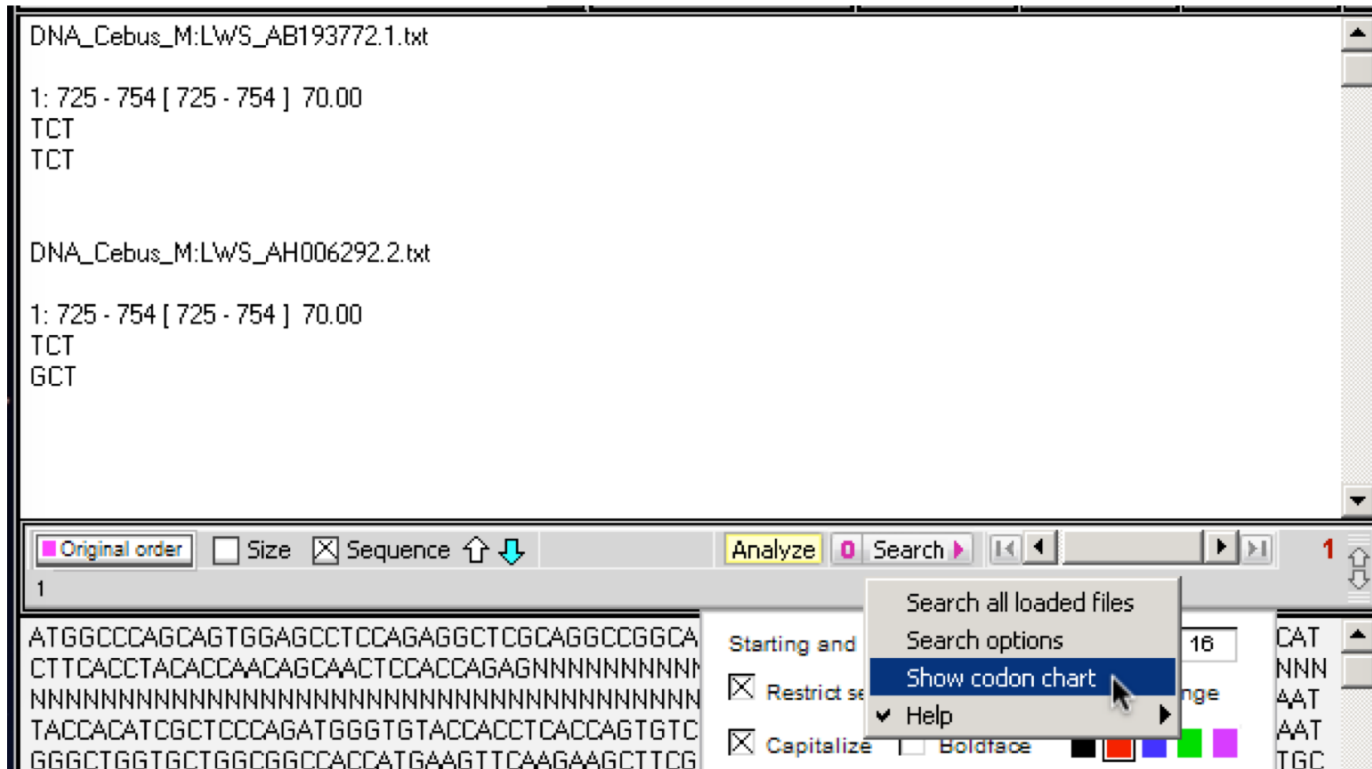


Then use the Search button and select **Search all loaded files**. This will re-search the files with this additional option checked.



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The summary of search results now shows only the three characters in the selected range (14 through 16), making it easier to see that TCT is present in the first file and GCT is present in the second file at location 180 in exon 3 of the opsin gene associated with color vision. Each of these triplets of letters represents a **codon**, so to determine which amino acids are associated with them we need a codon chart. Click the **Search** button and select **Show codon chart** as shown below.



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The codon chart indicates that **TCT** is the codon associated with the amino acid **Serine (S)**, whereas **GCT** is associated with **Alanine (A)**, so the individual monkeys from which these samples were taken had **S** and **A**, respectively, at the **180** position on exon 3 of the opsin gene. This has significance in terms of the wavelengths of colors that these two individuals could detect (see See [Color Vision Polymorphism in Wild Capuchins and Spider Monkeys in Costa Rica](#), also referenced on Slide 18).

The screenshot shows a software interface with a DNA sequence and a codon chart. The DNA sequence is as follows:

```

DNA_Cebus_M:LWS_AB193772.1.txt
1: 725 - 754 [ 725 - 754 ] 70.00
TCT
TCT

DNA_Cebus_M:LWS_AH006292.2.txt
1: 725 - 754 [ 725 - 754 ] 70.00
TCT
GCT
  
```

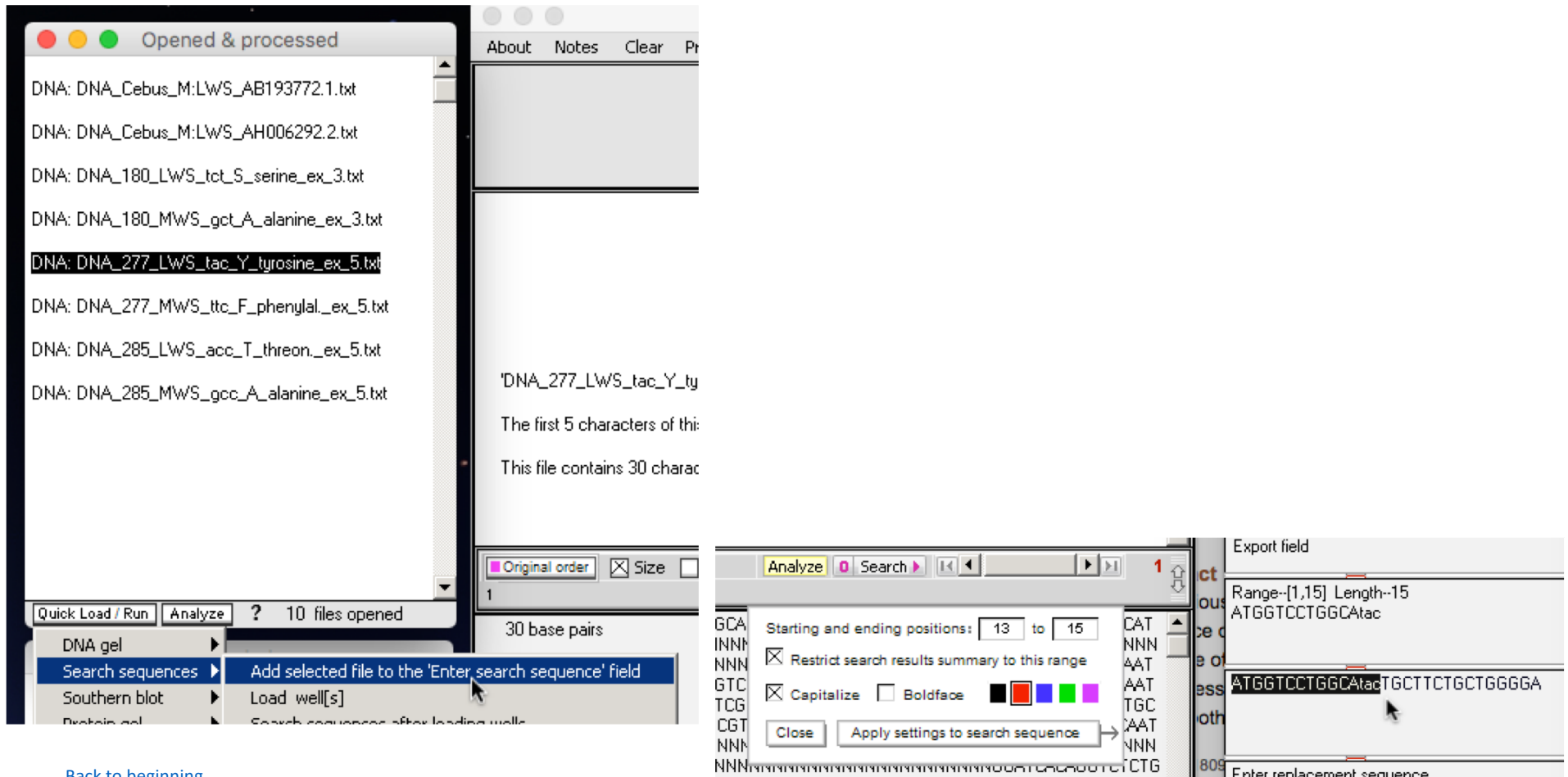
The codon chart is organized by the first base (T, C, A, G) and the second base (T, C, A). The amino acid for each codon is listed, along with its three-letter code and one-letter code.

First base	Second base		
	T	C	A
T	TTT F <i>Phenylalanine</i>	TCT S <i>Serine</i>	TAT Y <i>Tyrosine</i>
	TTC F	TCC S	TAC Y
	TTA L <i>Leucine</i>	TCA S	TAA stop
	TTG L	TCG S	TAG stop
C	CTT L	CCT P <i>Proline</i>	CAT H <i>Histidine</i>
	CTC L	CCC P	CAC H
	CTA L	CCA P	CAA Q <i>Glutamine</i>
	CTG L	CCG P	CAG Q
A	ATT I <i>Isoleucine</i>	ACT T <i>Threonine</i>	AAT N <i>Asparagine</i>
	ATC I	ACC T	AAC N
	ATA I	ACA T	AAA K <i>Lysine</i>
	ATG M <i>Methionine</i>	ACG T	AAG K
G	GTT V <i>Valine</i>	GCT A <i>Alanine</i>	GAT D <i>Aspartic acid</i>
	GTC V	GCC A	GAC D

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In the **Opened & processed window**, click on the line **DNA: DNA\_277\_LWS\_tac\_Y\_tyrosine\_ex\_5.txt** and use the **Quick Load/Run** button to select **Search sequences** -> **Add selected file to the 'Enter search sequence' field**. The search sequence associated with that file will appear in the second field from the bottom in the **Sequence analysis** window, as shown at right. Drag to determine the position of the lowercase letters **tac** (**13** through **15**), open the **Search options** box, enter these numbers as the starting and ending positions, and finally click **Apply settings to search sequence**.



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There were 90% and 80% matches, respectively, for the two files. An examination of the codon chart (not shown here) reveals that TAC is the codon for tyrosine (Y), and TTC is the codon for phenylalanine (F). These represent the amino acids at location 277 of exon 5 of the opsin gene, another important location for color vision in primates and other animals. If you repeat this procedure for location 285 on exon 5, using the search string for **DNA\_285\_LWS\_acc\_T\_threon\_ex\_5.txt**, the amino acids present at this location can be determined for both files.

Verify that for the monkey associated with AB193772.1, the amino acids at positions 180, 277, and 285 are S Y, and T, respectively. See [Color Vision Polymorphism in Wild Capuchins and Spider Monkeys in Costa Rica](#), the source of this data, for information relating to its significance. Question: What are the comparable amino acids at these positions in humans with full color vision, and humans that are colorblind?

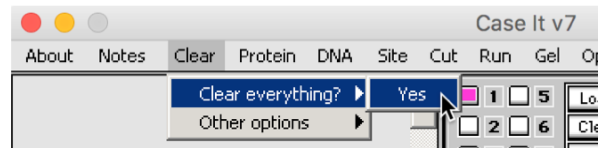
The screenshot displays a sequence analysis software interface. The main window is divided into several panes:

- Top Left:** Search results for two files:
  - DNA\_Cebus\_M:LWS\_AB193772.1.txt: 1: 1217 - 1246 [ 1217 - 1246 ] 90.00, TAC
  - DNA\_Cebus\_M:LWS\_AH006292.2.txt: 1: 1217 - 1246 [ 1217 - 1246 ] 80.00, TAC, TTC
- Bottom Left:** A DNA sequence with a search window from position 13 to 15. The sequence is:
 

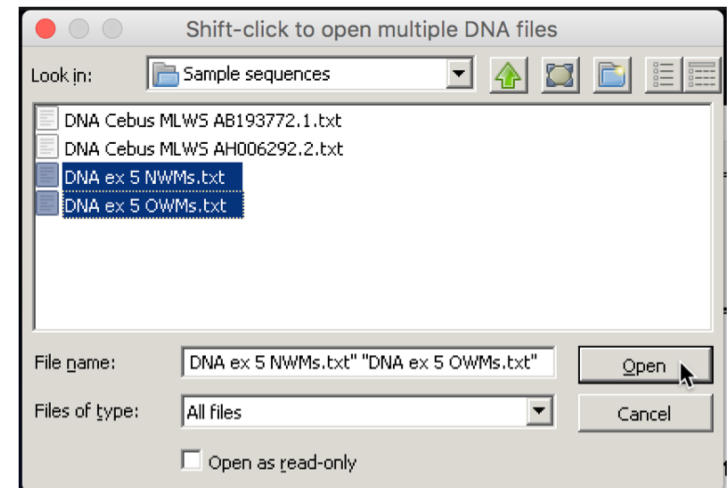
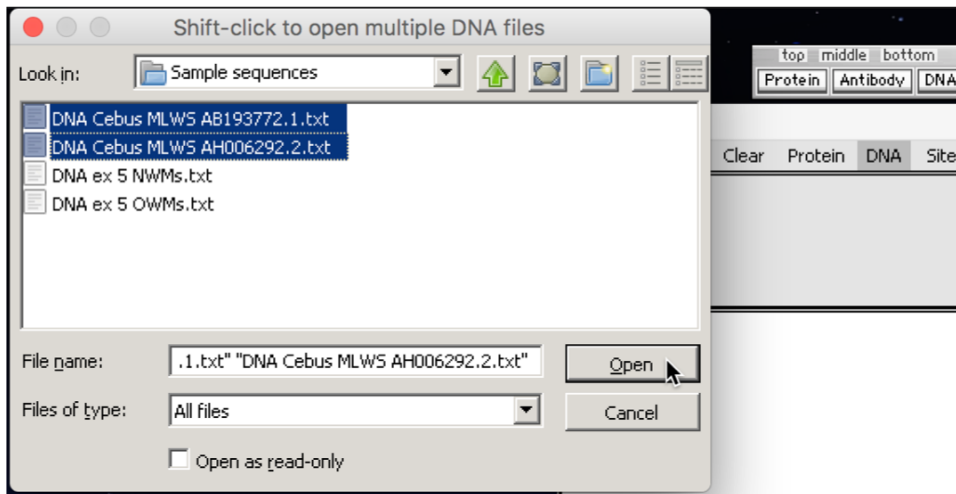
```
GTACCCCGGGGTGCAGCCTTACATGATCGTCCTCATGATCAC
CTCCAAGTGTGGCTGGCCATCCGAGCTNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
GAATCCACCCAGAAGGCAGAGAAGGAAGTGACACGCATGGT
CTACGCCTTCTTCGCATGCTTTGCTGCTGCCAACCCCTGGCTA
GCCAAAAGTGCCACTATCTACAACCCCATATCTATGTCTTTA
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNTTTCGAAACTGCATCTTGCAGCTTTTGGGAAGAAGGTTG
```
- Bottom Center:** A settings dialog box for the search window. It includes:
  - Starting and ending positions: 13 to 15
  - Restrict search results summary to this range
  - Capitalize  Boldface
  - Buttons: Close, Apply settings to search sequence
- Right Side:** A results pane titled 'ORIGINAL ORDER' showing:
  - Fragment number, location of search sequence within a particular fragment, [cumulative location], and % match
  - 1: 1217 - 1246 [ 1217 - 1246 ] 80.00
  - Export field
  - Range--[1,30] Length--30
  - ATGGTCCTGGCATACTGCTTCTGCTGGGGA
  - ATGGTCCTGGCATACTGCTTCTGCTGGGGA
  - ATGATCGTGACGTTCTGCGTCTGCTGGGGA

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Next, we'll demonstrate the enhanced PCR feature of Case It v7. Earlier versions of Case It required a 100% match of forward and reverse primers, but Version 7 allows you to set the match percent at any level. As an example, we'll use DNA sequences from a variety of New World and Old World monkeys, obtained from GenBank. Before beginning, use the Clear menu to clear all existing data from Case It v7.

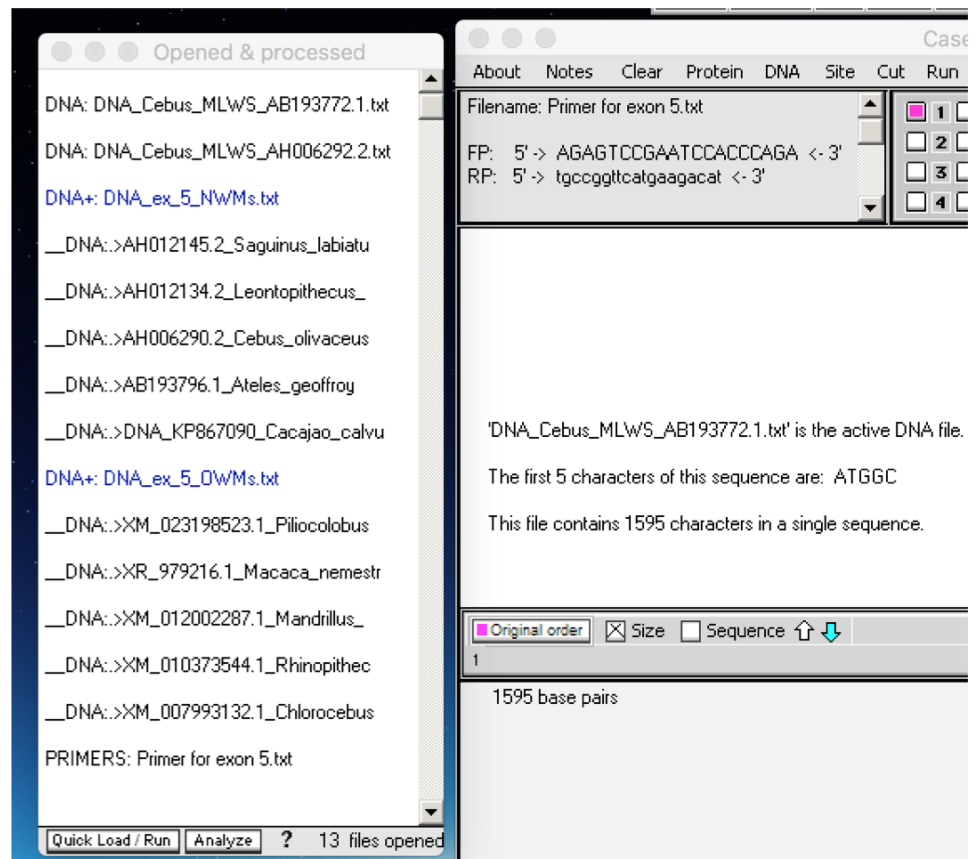
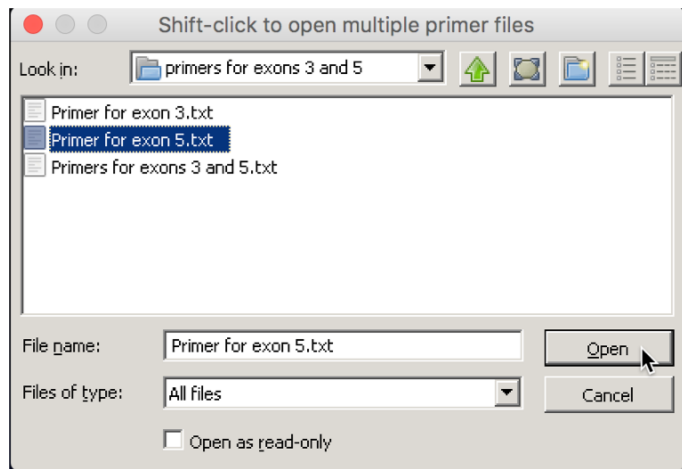


Click the **DNA** button and navigate through the folders **Case It v7 PC -> Cases -> Color Vision -> Sample sequences**. Shift-click to select the two DNA Cebus files used earlier, and click the **Open** button. Then click the **DNA** button again, select the other two files in the folder, and click the **Open** button. Note that you cannot shift click to open all four files simultaneously, since the second two files contain multiple DNA sequences each.



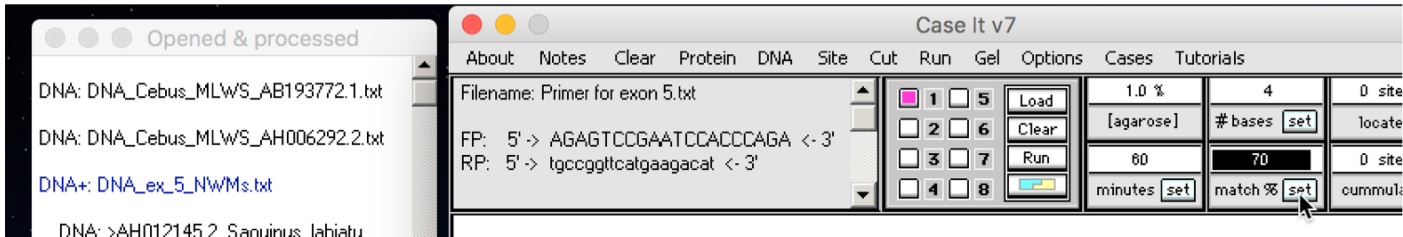
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Navigate to the **primers for exons 3 and 5** folder and open **Primer for exon 5.txt**. It will appear as a line at the bottom of the **Opened & processed** window after the DNA sequences opened previously. The blue lines indicate files that contain multiple DNA sequences, indicated by the underscore preceding each filename. The Cebus files contain one sequence per file.

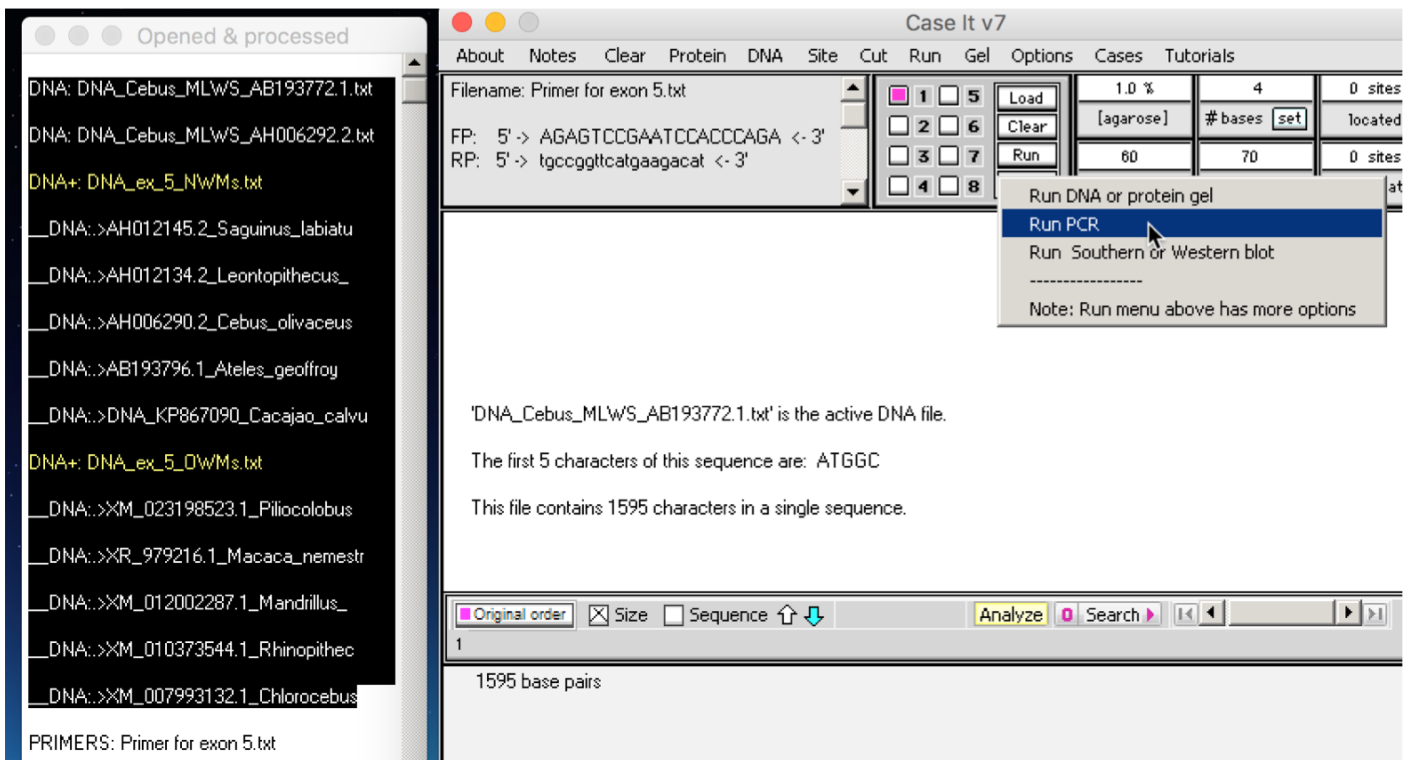


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Set the match % setting to 70, meaning that hits will occur for any match of 70% or higher (for both forward and reverse primers).



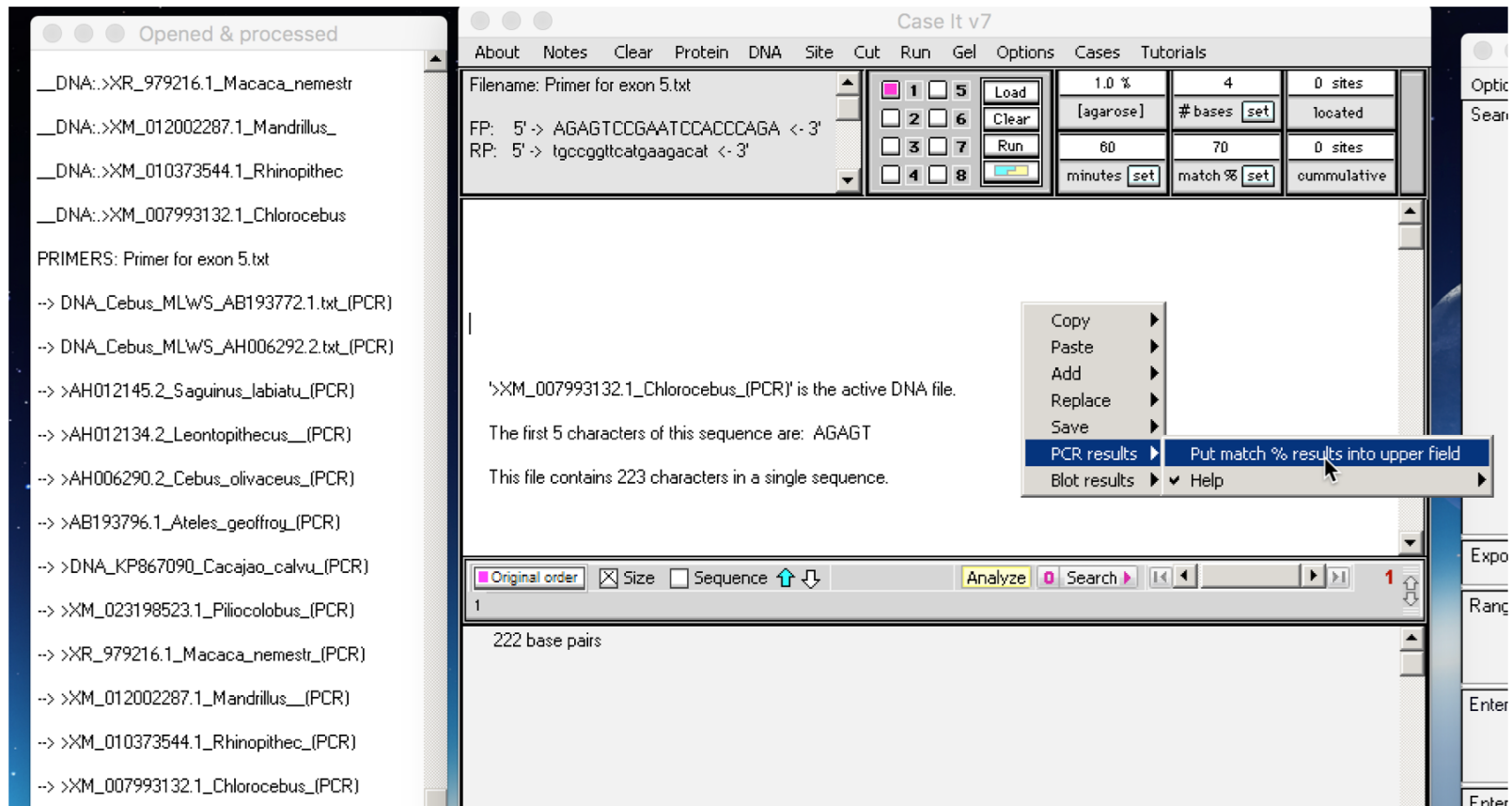
Shift click to select all the DNA files, making sure not to select the **Primers** file at the bottom, and then use the **Run** button and select **Run PCR**.



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New lines appear in the Opened & processed window, each representing a PCR product (designated by the arrow preceding the filename). To see a summary of results shown the percentage for each hit, **right-click** on the white upper field and select **PCR results -> Put match % in the upper field**.

Note: most fields in Case It have associated pop-up menus, activated by right-clicking.



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One of the matches is shown below, with a 95% match for the forward primer and a 94.74 match for the reverse primer. You would need to scroll in the field to see all of the results, so right-click to **Save** them to the Notepad of Case It. You can also **Copy** the results to the clipboard, for pasting into another application.

The screenshot shows a software window titled "Case It v7" with a menu bar (About, Notes, Clear, Protein, DNA, Site, Cut, Run, Gel, Options, Cases, Tutorials). Below the menu bar is a control panel with a grid of buttons (1-8), "Load", "Clear", "Run", and "minutes" (with a "set" button). To the right of the control panel are input fields for "1.0 %", "4", "0 sites", "[agarose]", "# bases" (with a "set" button), "located", "60", "70", "0 sites", "minutes" (with a "set" button), "match %" (with a "set" button), and "cumulative".

The main display area shows the following text:

```
Filename: Primer for exon 5.txt
FP: 5' -> AGAGTCCGAATCCACCCAGA <- 3'
RP: 5' -> TGCCGGTTCATGAAGACAT <- 3'
>XM_007993132.1_Chlorocebus
[FP match % , RP match %]
["95.00 FP", "94.74 RP"]
AGAGTCCGAATCCACCCAGA-FP
AGAGTCTGAATCCACCCAGA-DNA
ATGTCTTCATGAACCGGCA-RP
ATGTCTTTATGAACCGGCA-DNA
PCR product 1 length = 222
.....
```

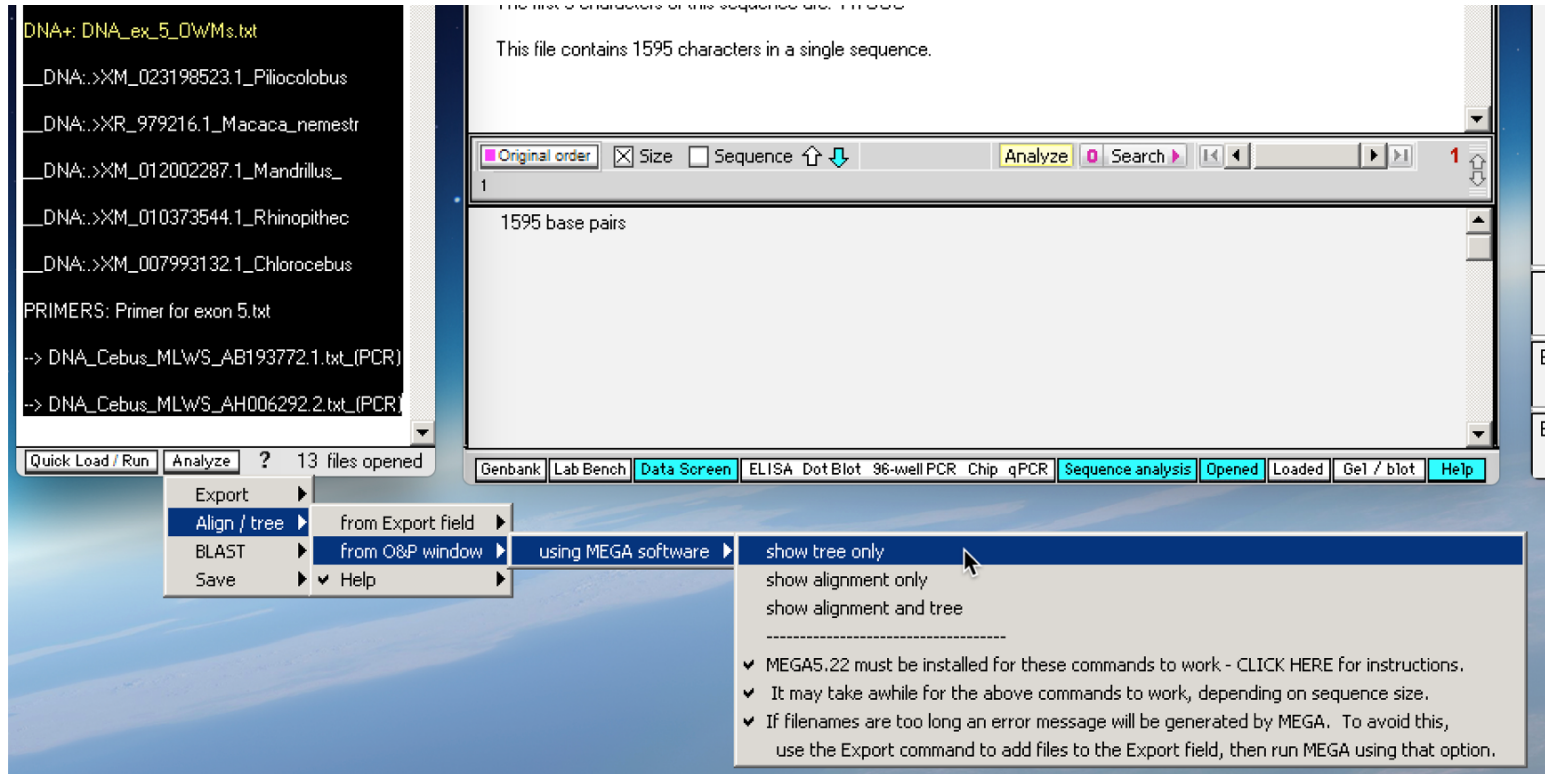
A context menu is open over the sequence, with options: Copy, Paste, Add, Replace, Save, PCR results, Blot results. The "Save" option is selected, showing a sub-menu with options: "Entire contents of upper field to Notepad" (selected), "Save all sequences in upper field of main window", "Save a specific sequence in lower field of main window", ".....", and "IMPORTANT: Rename files every time you save them -- [do not replace an existing file with a file of the same name]".

At the bottom of the main display area, there is a table with columns "Original order", "Size", and "Sequence". The first row shows "1" in the "Original order" column and "222 base pairs" in the "Size" column.

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To create a phylogenetic tree of this data with one click, **shift-click** to highlight all of the PCR products, then use the **Analyze** button and select **Align/tree -> from O&P window -> using MEGA software -> show tree only...**

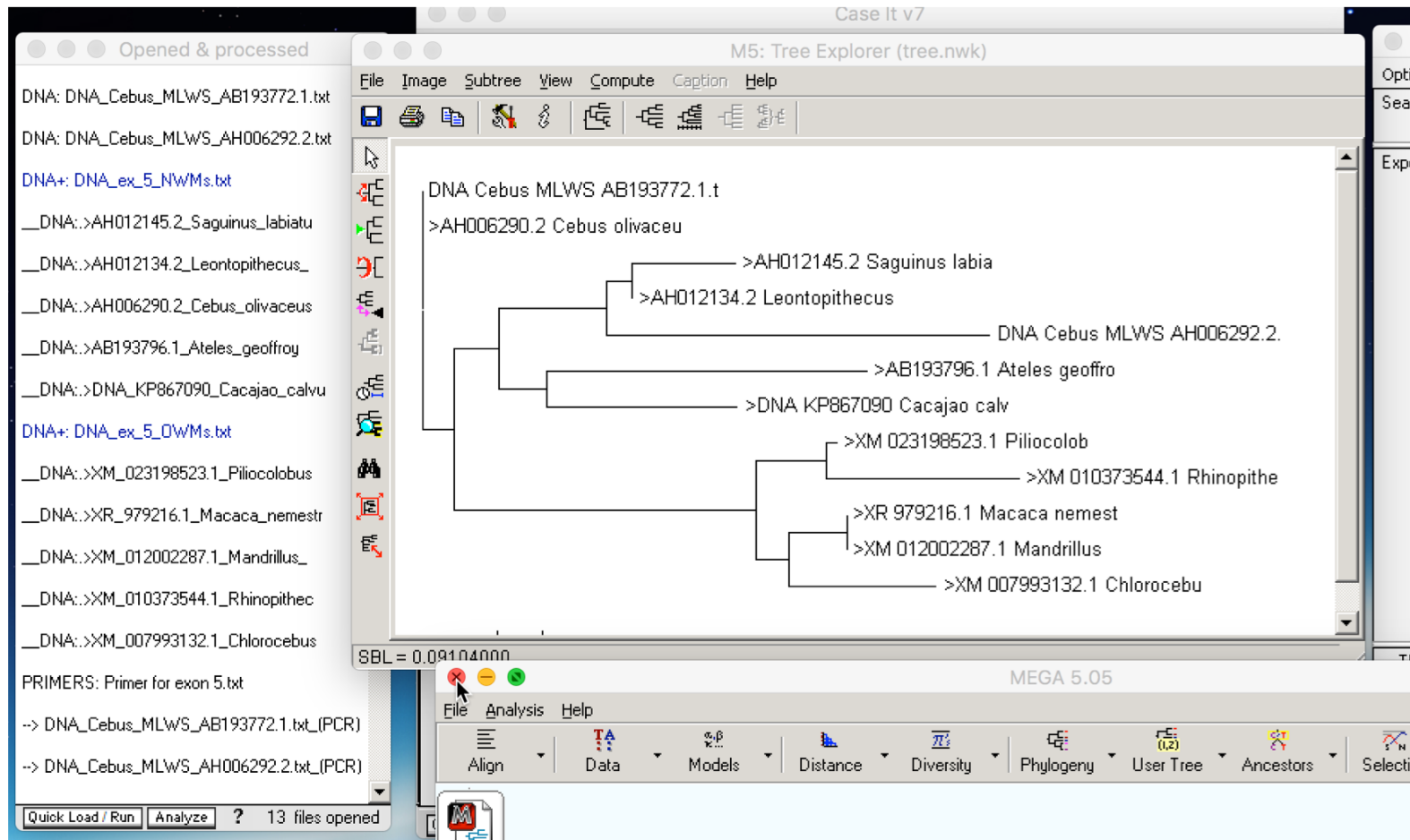
Note: This assumes that you have separately downloaded the MEGA executable and put it in the MEGA folder of Case It – for details, see the tutorial **Installing MEGA software for use with Case It.**



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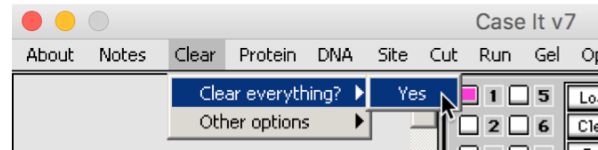


...and in a few seconds the tree appears. Case It also copy selected PCR products to the the clipboard and open two web-based sites for aligning sequences and building trees, the **MABL** site and the **MAFFT** site. See the **Bioinformatics** tutorial for details.

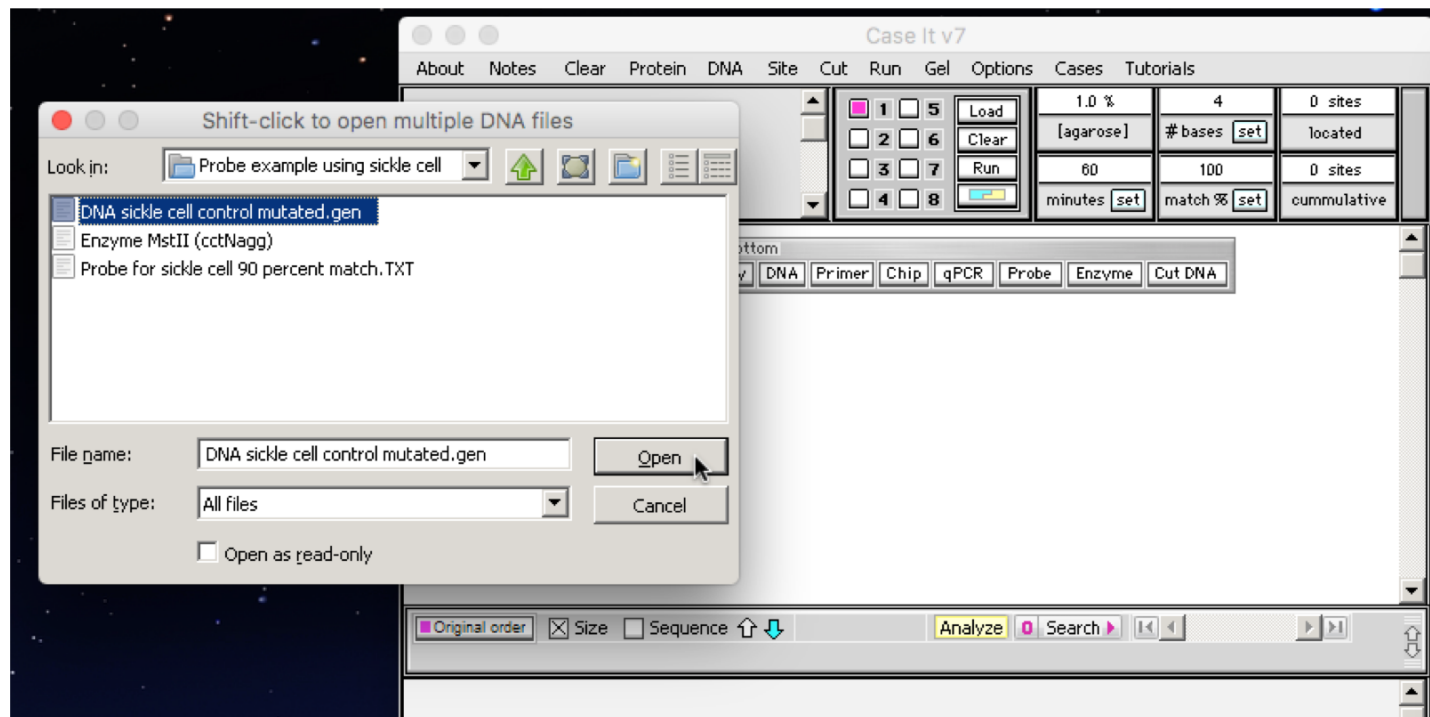


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Before demonstrating set search parameters for Southern and dot blots, use the Clear menu and select **Clear everything?** -> **Yes**.



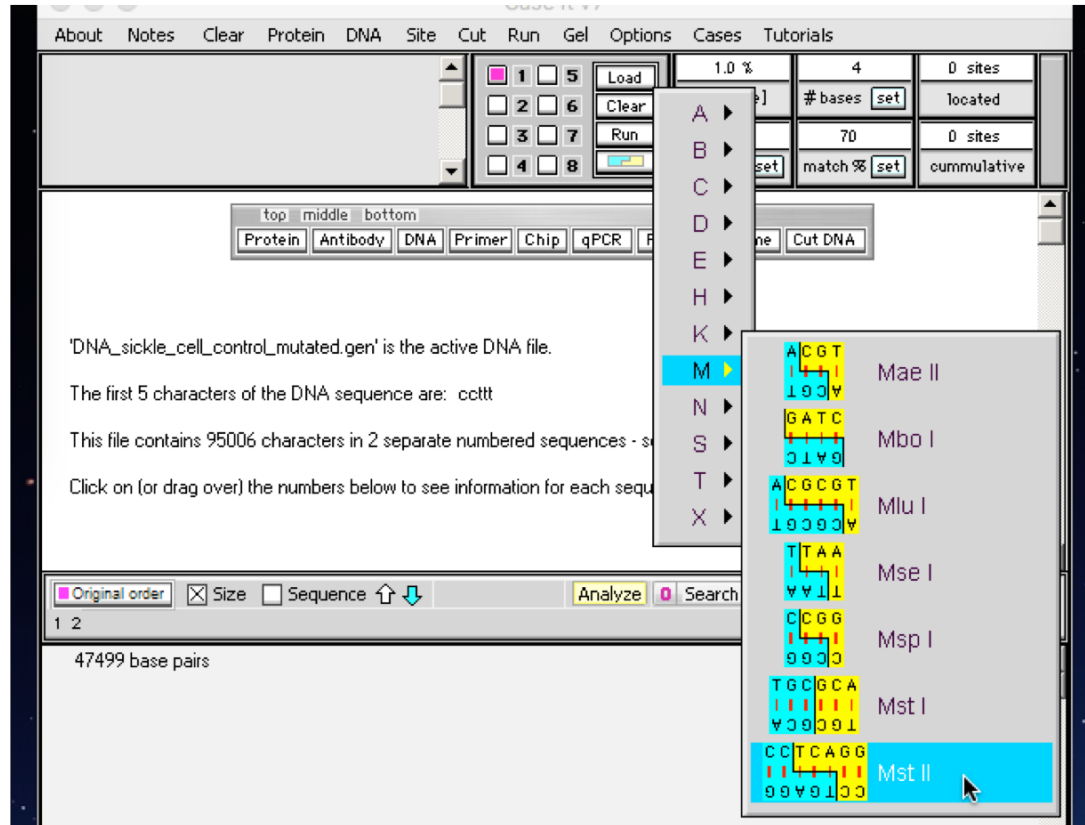
Click on the DNA button of the silver button bar, then navigate through the folders **Case It v7 PC -> Cases -> Test sequences -> DNA for digestion -> Probe example using sickle cell** and open the file **DNA sickle cell control mutated.gen**.



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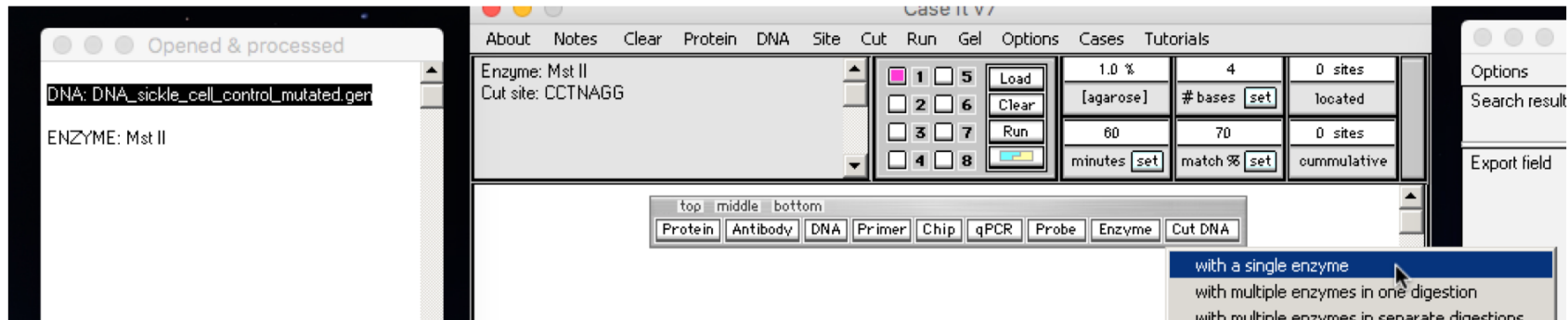
Before running a Southern blot, we need the restriction enzyme Mst II to digest the DNA sequence. Click the **button with the blue/yellow symbol** (located under the Run button) and select **M -> Mst II**.

Note: This file could also be accessed by clicking the Enzyme button, then selecting that file from the currently active folder.

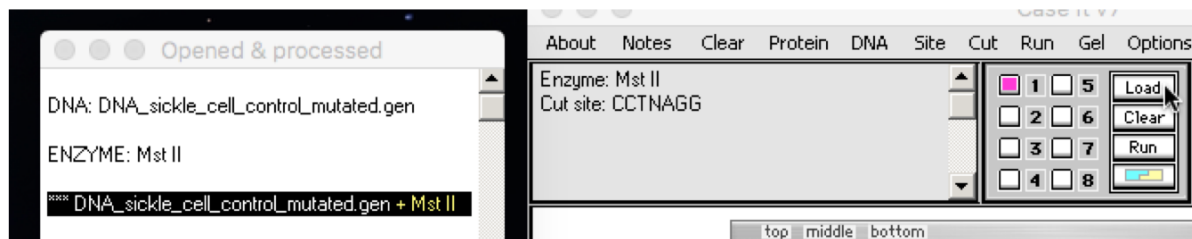


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Click the **Cut DNA** button on the silver button bar and select **with a single enzyme**.

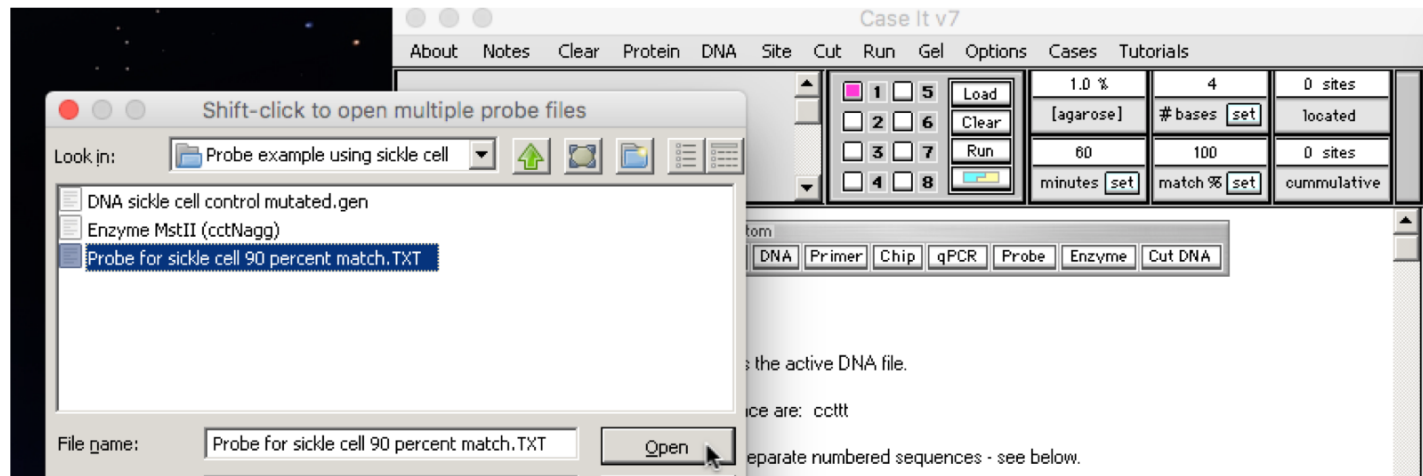


The digested file appears as a new line in the Opened & processed window, designated by a \*\*\* prefix and a + Mst II suffix. Click this line and then click the Load button to load the digested sequence into well one.



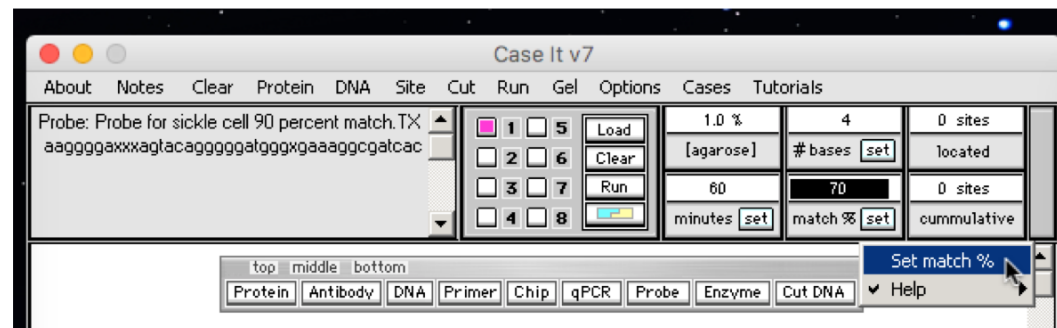
[Back to beginning](#)

Click on the **Probe** button of the silver button bar and open the file **Probe for sickle cell 90 percent match**.



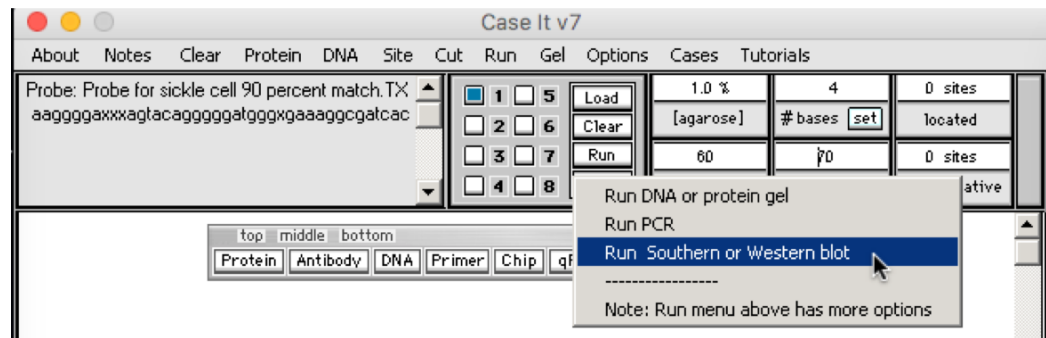
Change the number in the match% field to **70**, then click the **Set match %** button.

Note: Settings below 50% can cause the program to temporarily freeze and may give unpredictable results.

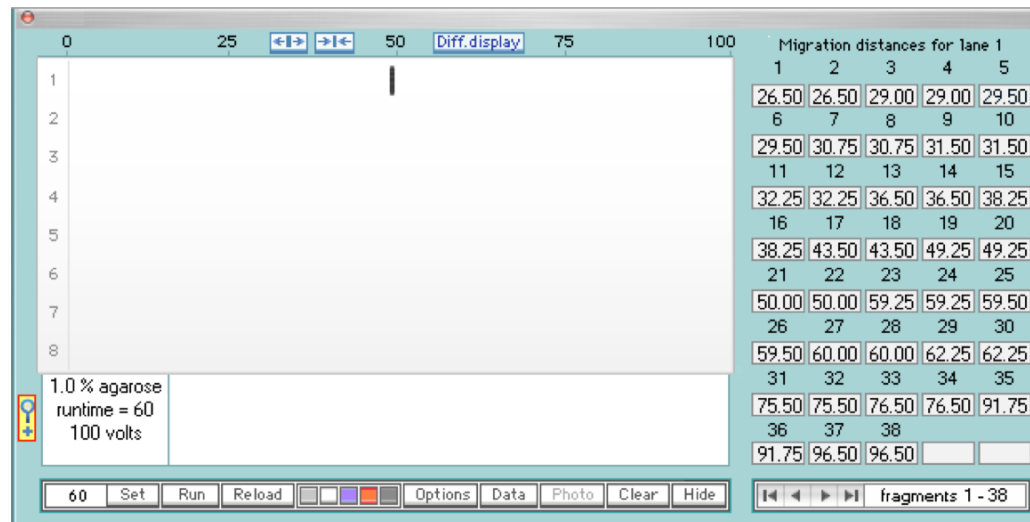


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Click the **Run** button and select **Run Southern or Western blot**.

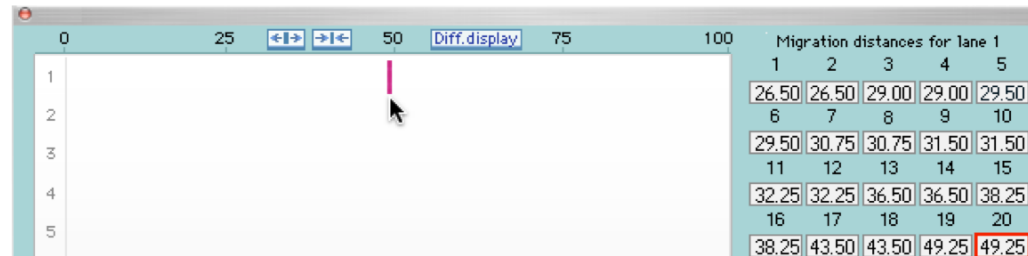


A Southern blot will appear with a single fragment showing, representing a fragment that bound to the probe at this match % setting. Note that there are 38 migration distances showing in the table to the right, indicating that other fragments are not visible since they did not bind to the probe.

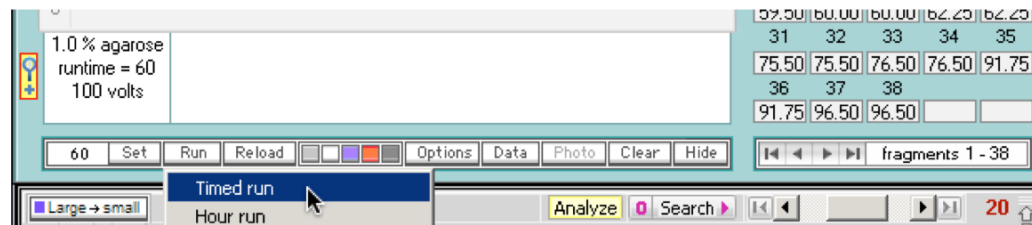


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Click on the fragment to turn it from black to red, and note that the fragment was the 20<sup>th</sup> one on the gel prior to running the blot procedure.



To see the original gel upon which the blot is based, click on the **Run** button and select **Timed run**.



“Ghost fragments” will appear on the gel, that would not be visible in reality without staining.



To stain the fragments, click the blue button (or the orange or black buttons).

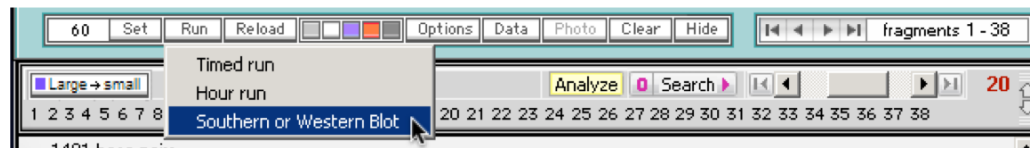


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All fragments in the lane will turn blue, indicating that they have been stained.



To re-run the blot to see which fragments were bound to the probe, use the **Run** button and select **Southern or Western blot...**



...and the fragment bound to the probe appears.



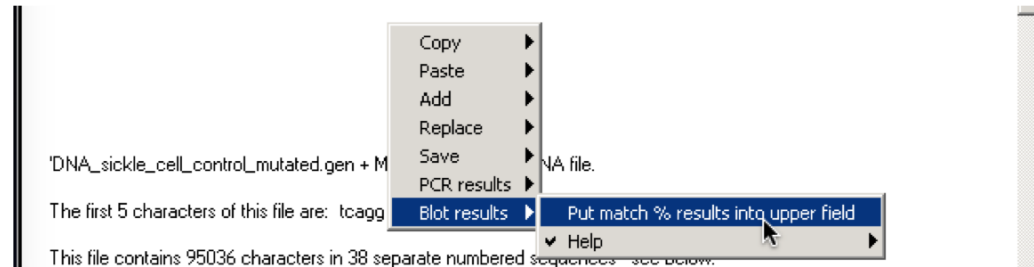
Temporarily hide the blot by clicking the Hide button.



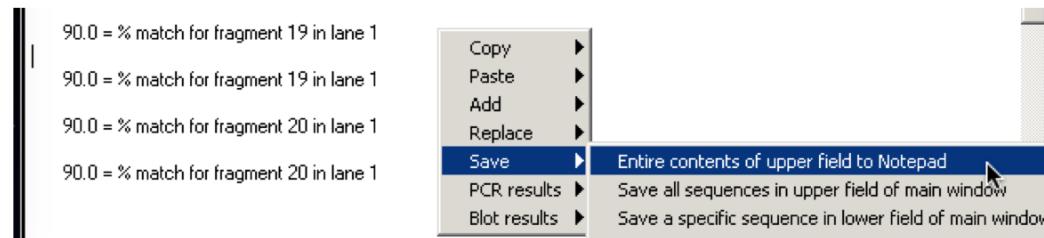
[Back to beginning](#)



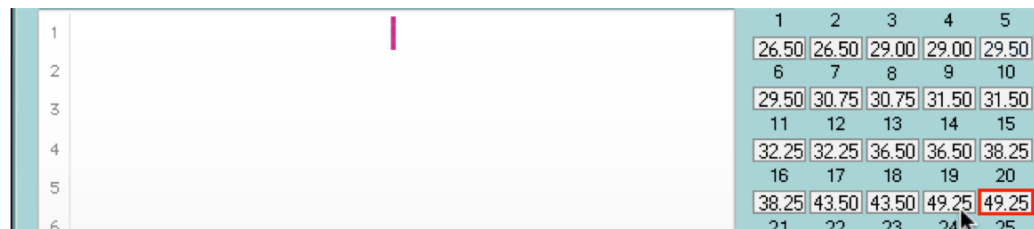
Right-click on the upper field and select **Blot results -> Put match % results into upper field.**



Actual match percentages (90%) appear in the field, indicating that both fragments 19 and 20 were bound to the probe. Note that the second and fourth lines are duplicates of the first and third lines, so they can be ignored. These results can be copied or saved by right-clicking.

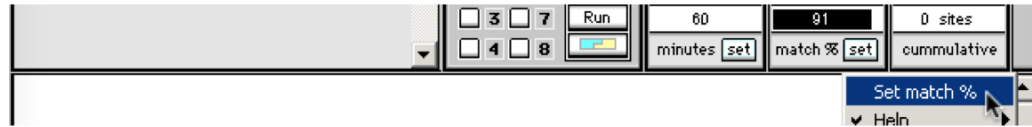


Since only one band appeared after the Southern blot, fragments 19 and 20 must be at the same location, which can be verified by looking at the migration distances and clicking on the boxes associated with fragments 19 and 20.



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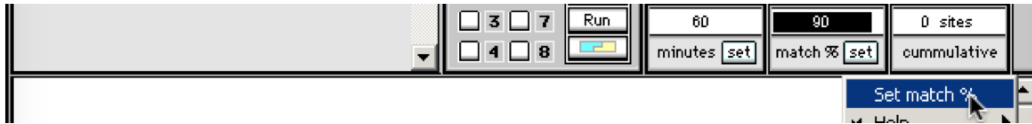
To make sure that the probe match routine is working properly, set the match % to **91**...



...and no match will occur since 91 is higher than 90, the actual percentage required for a match with this probe and sequence.



Set the match % back to **90**...



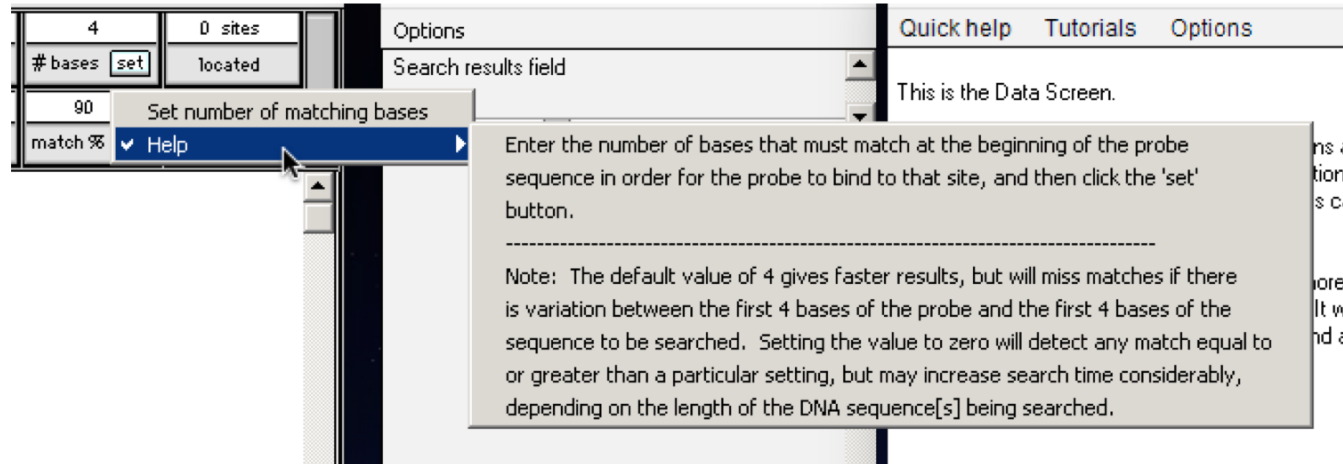
...and the black band reappears, indicating that the procedure is working properly.



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The previous probe searches assumed that there was a perfect match between the first 4 characters of the probe sequence and the hit site on the DNA sequence being searched, as shown in the **# bases** field below. This was done to speed up searching for existing Case It cases, so that is the default setting for the software. The value can be reduced down to zero, which is more realistic if you are using your own probe for research or case development purposes, but search times may increase substantially as described in the **Help** message below.

Important: The **# bases** box only applies to the Southern blot and dot blot routines. Values in this box have no effect on the general search and PCR routines described earlier in this tutorial, as those routines always search from the beginning to the end of sequences one character at a time, for greater realism and usefulness for research purposes. [Lower **match %** settings will slow down these routines, depending on the size of sequences being searched.]



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## Obtaining sequences for research purposes

A good place to start a research project is to find an article with published forward and reverse primers, for example the one below that analyzed exon 3 and exon 5 of the opsin gene, a gene involved with color vision in primates and many other animals ([click here for a link to the full article.](#))

Proc Biol Sci. 2016 Apr 13; 283(1828): 20160067. PMID: PMC4843651  
doi: [10.1098/rspb.2016.0067](https://doi.org/10.1098/rspb.2016.0067)

**Highly polymorphic colour vision in a New World monkey with red facial skin, the bald uakari (*Cacajao calvus*)**

[Josmael Corso](#),<sup>1</sup> [Mark Bowler](#),<sup>2,3</sup> [Eckhard W. Heymann](#),<sup>3</sup> [Christian Roos](#),<sup>4</sup> and [Nicholas I. Mundy](#)<sup>5</sup>

[Author information](#) ▶ [Article notes](#) ▶ [Copyright and License information](#) ▶

Scroll down to the Materials and Methods section of the article until you find the section below. The four DNA sequences are:

Primer3CCF2: forward primer for exon 3    Primer3CCR2: reverse primer for exon 3  
Primer5CCF2: forward primer for exon 5    Primer5CCR3: reverse primer for exon 5

**(c) Genotyping of the X-linked opsin locus**

We designed oligonucleotide primers to amplify exons 3 and 5 of the LWS/MWS opsin gene from an alignment of existing NWM data from Genbank: Exon 3, Primer3CCF2 5'-CTCTGGTCCCTGGCCATCATT-3' and Primer3CCR2 5'-CCCCTTACCTGCTCCAACCAA-3'; Exon 5, Primer5CCF2 5'-AGAGTCCGAATCCACCCAGA-3' and Primer5CCR3 5'-TGCCGGTTCATGAAGACAT-3'. PCR reactions contained approximately 50 ng DNA template, 2.5

Nucleotides at position 180 on exon 3 and positions 277 and 285 on exon 5 help determine whether bald uakaris have dichromatic or trichromatic color vision, a characteristic with significant evolutionary and ecological consequences. [See the [Monkey Opsin section of the Evo-Ed website](#) for a detailed explanation of the molecular biology of color vision, as it relates to the evolution and ecology of New World and Old World primates.]

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The first step is to create a Primer file that can be read by Case It. Highlight the **forward primer sequence for exon 5** and right-click to copy it to your computer's clipboard. Note: Although Case It v7 is capable of multiplex PCR, we will only use the primers for exon 5 for this example.

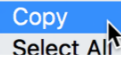
### (c) Genotyping of the X-linked opsin locus

We designed oligonucleotide primers to amplify exons 3 and 5 of the LWS/MWS opsin gene from an alignment of existing NWM data from Genbank: Exon 3, Primer3CCF2

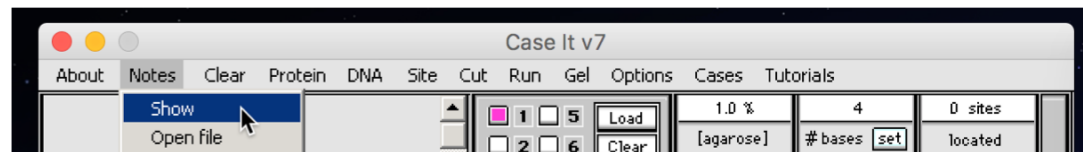
5'-CTCTGGTCCCTGGCCATCATT-3' and Primer3CCR2 5'-CCCCTTACCTGCTCCAACCAA-3';

Exon 5, Primer5CCF2 5'-AGAGTCCGAATCCACCCAGA-3' and Primer5CCR2

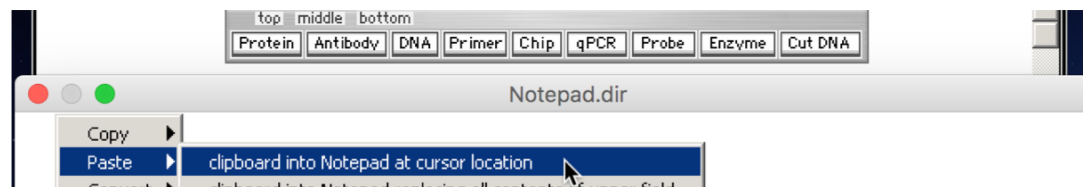
5'-TGCCGGTTCATGAAGACAT-3'. PCR reactions contain



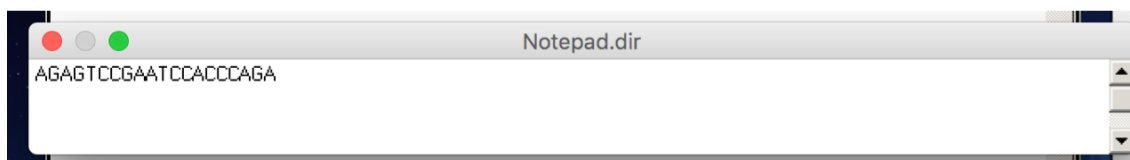
Click the **Notes** menu of Case It v7 and select **Show**, which causes a simple **Notepad** text editor to appear.



Right-click in the Notepad and select **Paste -> clipboard into Notepad at cursor location**.



The forward primer for exon 5 is now the first line in the Notepad.

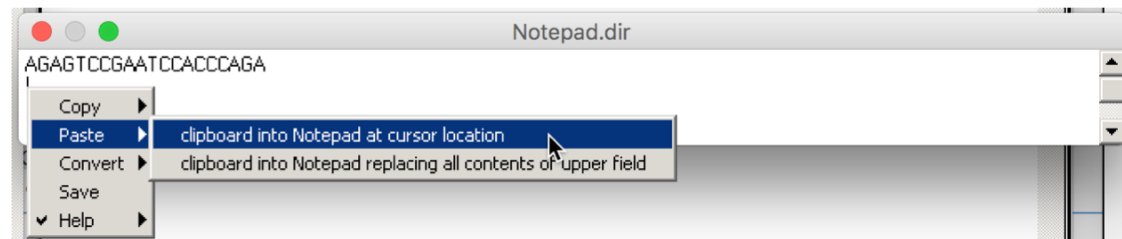


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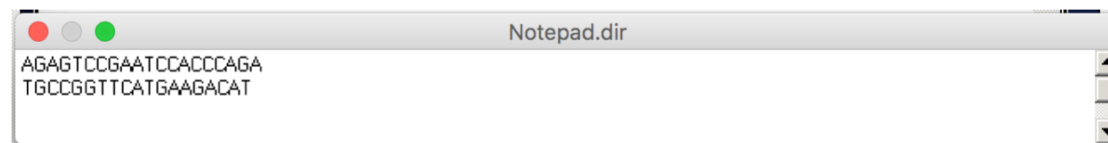
Go back to the article and select and copy the **reverse primer** sequence for exon 5.

alignment of existing NWM data from Genbank: Exon 3, Primer3CCF2  
5'-CTCTGGTCCCTGGCCATCATT-3' and Primer3CCR2 5'-CCCCTTACCTGCTCCAACCAA-3'; Exon  
5, Primer5CCF2 5'-AGAGTCCGAATCCACCCAGA-3' and Primer5CCR3  
5'-TGCCGGTTCATGAAGACAT-3'. PCR reactions contained approximately 50 ng DNA template, 2.5  
mM each dNTP, 1× PCR buffer, 1.0 U Taq polymerase, 0.25 μM each

Position your cursor at the beginning of the second line of the Notepad, then **Paste** in the reverse primer sequence so that the forward primer is line 1 of the file, and the reverse primer is line 2.

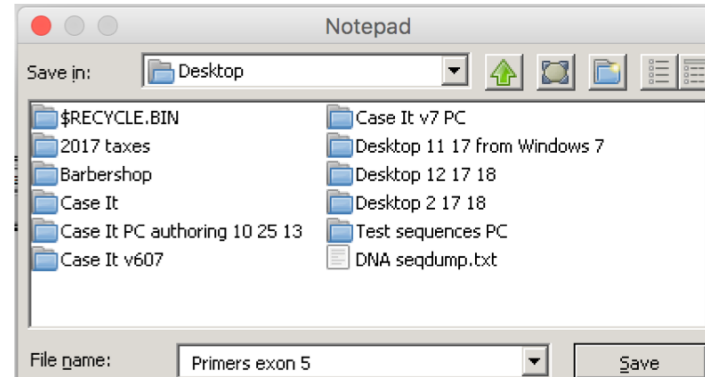
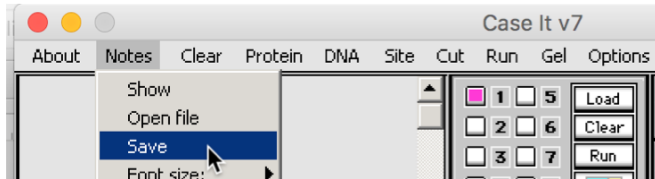


Additional forward and reverse primers could be pasted in, for example the FP and RP for exon 3, but we will not do this here. The general rule is to add each primer set as additional lines in the file (e.g. lines 3 and 4 for the next set, lines 4 and 5 for the set after that, and so forth.) See the STD case for an example of how multiplex PCR works in Case It v7.

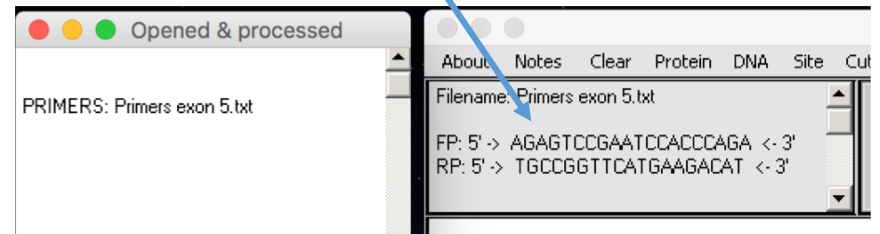
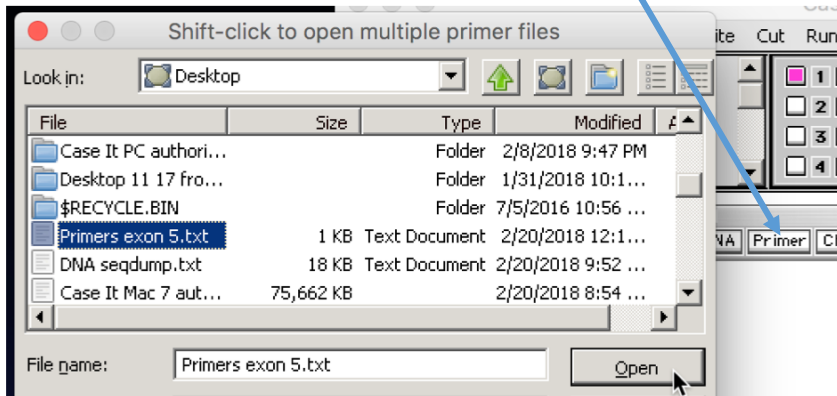


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Use the **Save** command of the **Notes** menu, and save the file after renaming it **Primers exon 5** to the desktop or another location on your computer. Note that files containing primers must always include the word **Primers** at the beginning of the filename.

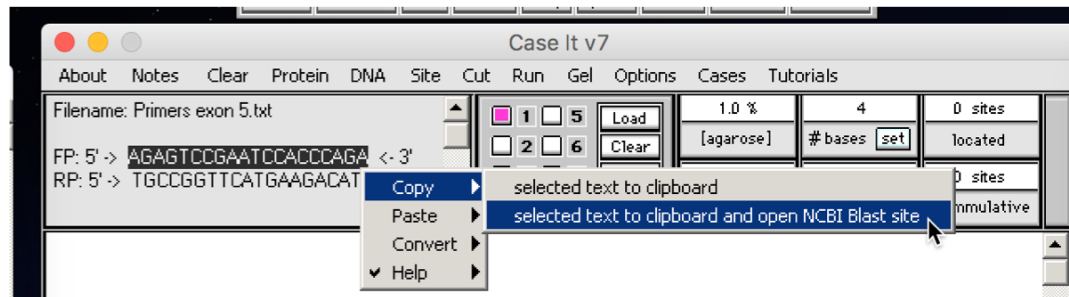


Click the **Primer** button on the silver button bar and open the **Primer exon 5.txt** file that you just saved. It will appear in the Opened & processed window, and also in the gray field of the main window of Case It.

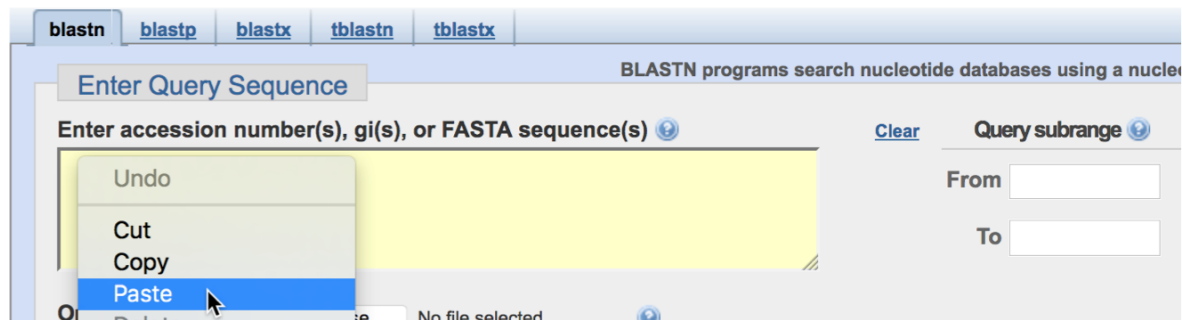


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We now need to obtain DNA sequences that contain the forward primer sequence for exon 5 of the opsin gene. **Double-click** on the forward primer in the gray field to highlight it, then **right-click** in the field and select **Copy -> selected text to clipboard and open NCBI Blast site**.



Your default web browser will automatically open to the BLASTN page of the NCBI web site. **Right-click** in the **Enter Query Sequence** box on this page, then **paste** the forward primer sequence into the box. **You may have to paste more than once** for this to work.



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





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Once the page appears showing results, scroll down on the page and select the first 10 hits (we are doing this strictly to illustrate the procedure; in reality you would want to carefully examine hits and select those most appropriate for the hypothesis you are testing).

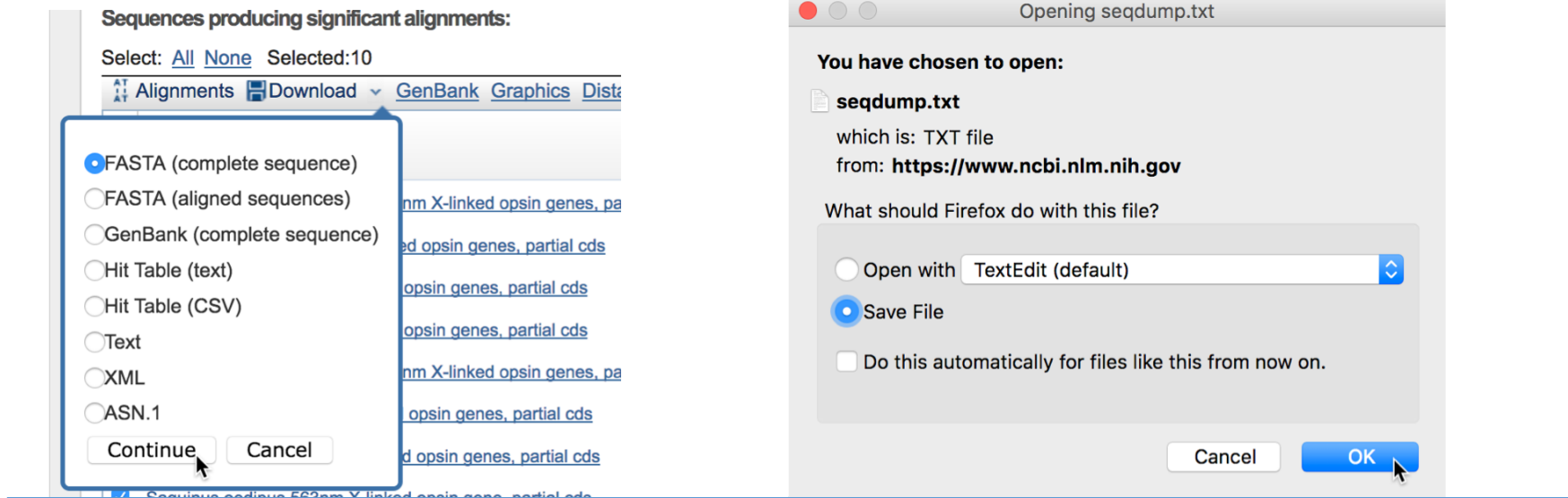
**Sequences producing significant alignments:**

Select: [All](#) [None](#) Selected:10

 <a href="#">Alignments</a>  <a href="#">Download</a> <a href="#">GenBank</a> <a href="#">Graphics</a> <a href="#">Distance tree of results</a>				
	Description	Max score	Total score	Query cover
<input checked="" type="checkbox"/>	<a href="#">Leontopithecus chrysomelas 563nm X-linked opsin genes, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Callithrix pygmaea 563nm X-linked opsin genes, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Callimico goeldii 563nm X-linked opsin genes, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Callimico goeldii 543nm X-linked opsin genes, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Leontopithecus chrysomelas 556nm X-linked opsin genes, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Callithrix jacchus 556nm X-linked opsin genes, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Callithrix geoffroyi 563nm X-linked opsin genes, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Saguinus oedipus 563nm X-linked opsin gene, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Saguinus midas 556nm X-linked opsin gene, partial cds</a>	42.1	42.1	100%
<input checked="" type="checkbox"/>	<a href="#">Saguinus fuscicollis 563nm X-linked opsin gene, partial cds</a>	42.1	42.1	100%

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Click the **Download** button using the default setting (download the complete FASTA sequence). Click the **Continue** button, select **Save File**, and click **OK**. You will then be able to save the file to your desktop or another location on your computer.

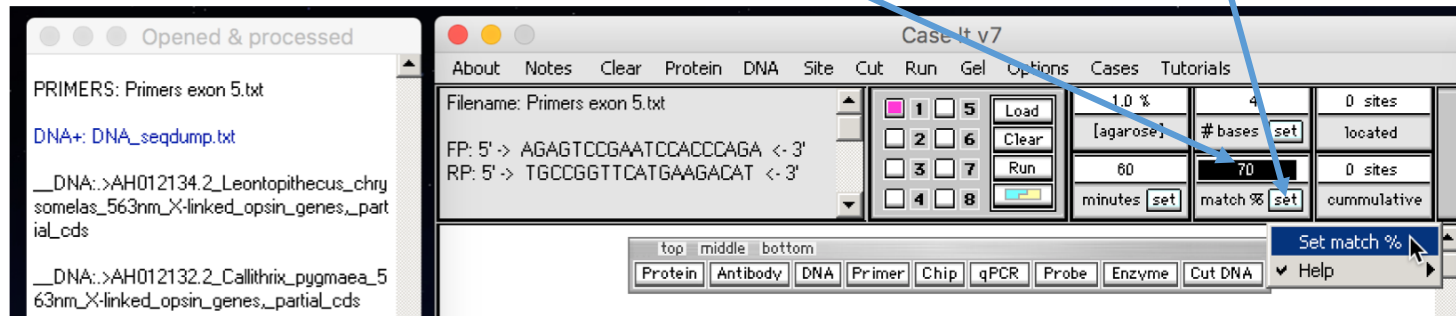


The filename will be saved by the NCBI site with the name **seqdump.txt**. After locating the downloaded file, **change the name of the file**, making sure to begin the filename with the letters **DNA**. Otherwise, Case It v7 will not recognize it as a DNA file. In this example, the file has simply been name **DNA seqdump.txt**, but you should give it a more descriptive name that retains the DNA prefix.

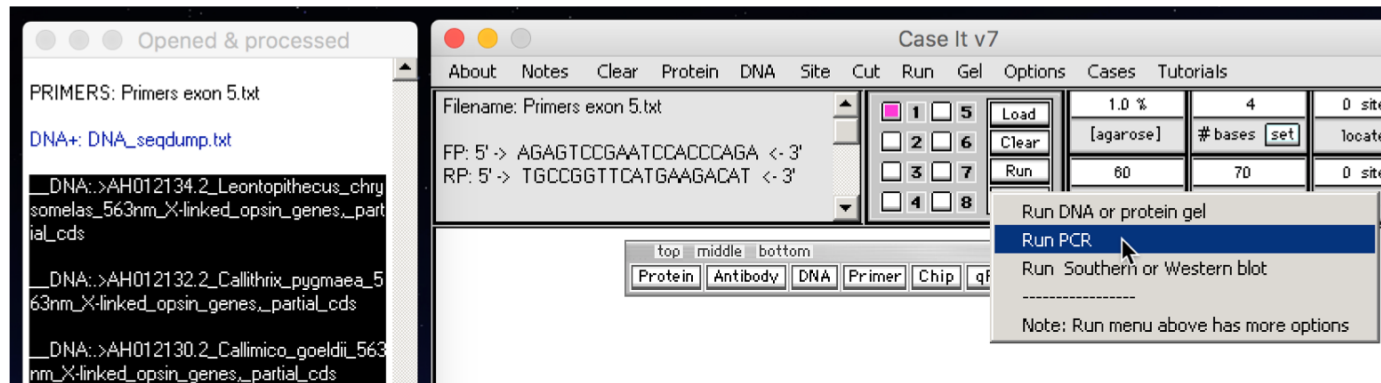


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Use the **DNA** button on the silver button bar to open the file. Enter **70** in the **match %** field and click the **Set** button.



Shift-click in the **Opened & processed** window to select all 10 lines (only 3 of which are shown here), then click the **Run** button and select **Run PCR**.



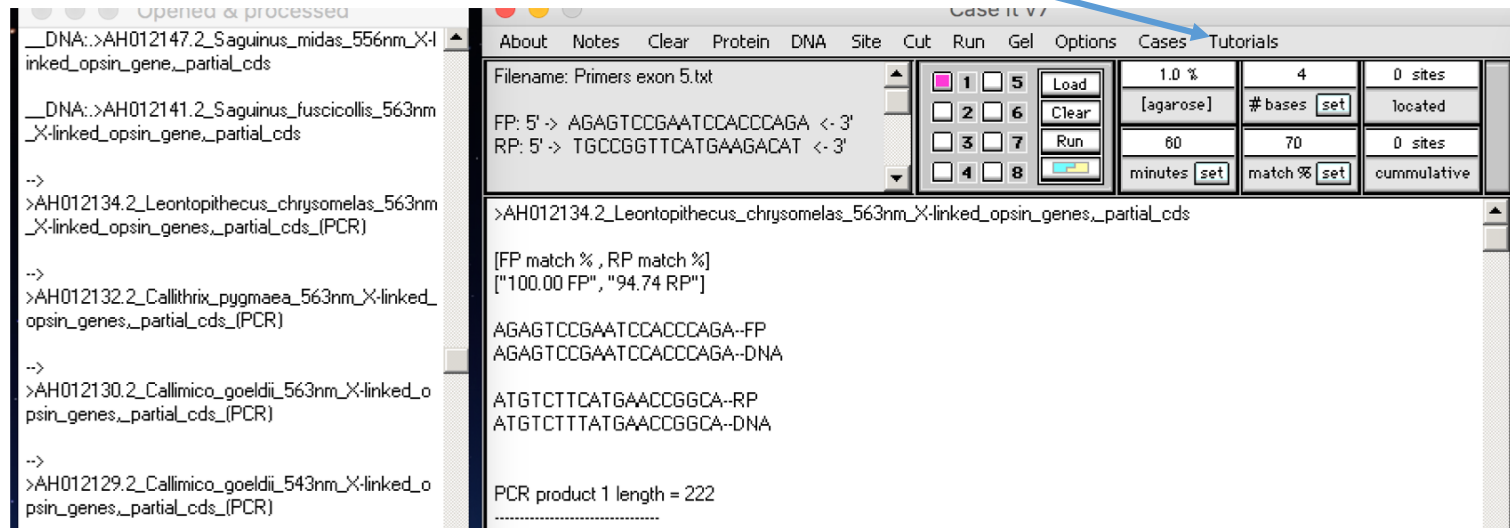
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PCR products appear in the O&P windows, designated by arrows at the beginning of each filename. **Right-click** on the large white field and select **PCR results -> Put match % results into upper field**.

The screenshot displays the Case It v7 software interface. On the left, a window titled "Opened & processed" lists several PCR products, each starting with an arrow and a filename. The main window, titled "Case It v7", shows a detailed view of a selected product. The "Filename" field contains "Primers exon 5.txt". The "FP" (Forward Primer) is "5' -> AGAGTCCGAATCCACCCAGA <- 3'" and the "RP" (Reverse Primer) is "5' -> TGCCGGTTTCATGAAGACAT <- 3'". The main text area shows the first 5 characters of the sequence: ">AH012141.2\_Saguinu...". A context menu is open over this text, with the option "Put match % results into upper field" highlighted. The bottom status bar shows "222 base pairs" and "1" in a small box. The bottom navigation bar includes buttons for "Genbank", "Lab Bench", "Data Screen", "ELISA", "Dot Blot", "96-well PCR", "Chip", "qPCR", "Sequence analysis", "Opened", "Loaded", "Gel / blot", and "Help".

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For the first PCR product, there was a 100% match for the forward primer (FP), and a 94.74% match for the reverse primer. Note that the forward primer in the white field is the same as the forward primer in the gray field, whereas the reverse primer (RP) in the white field is the **reverse complement** of the reverse primer sequence in the gray field. When Case It v7 opens primer files, it automatically converts the reverse primer into its reverse complementary sequence. To see why this is necessary, click the **Tutorials** menu and watch the video tutorial called **Show PCR primer sites**.

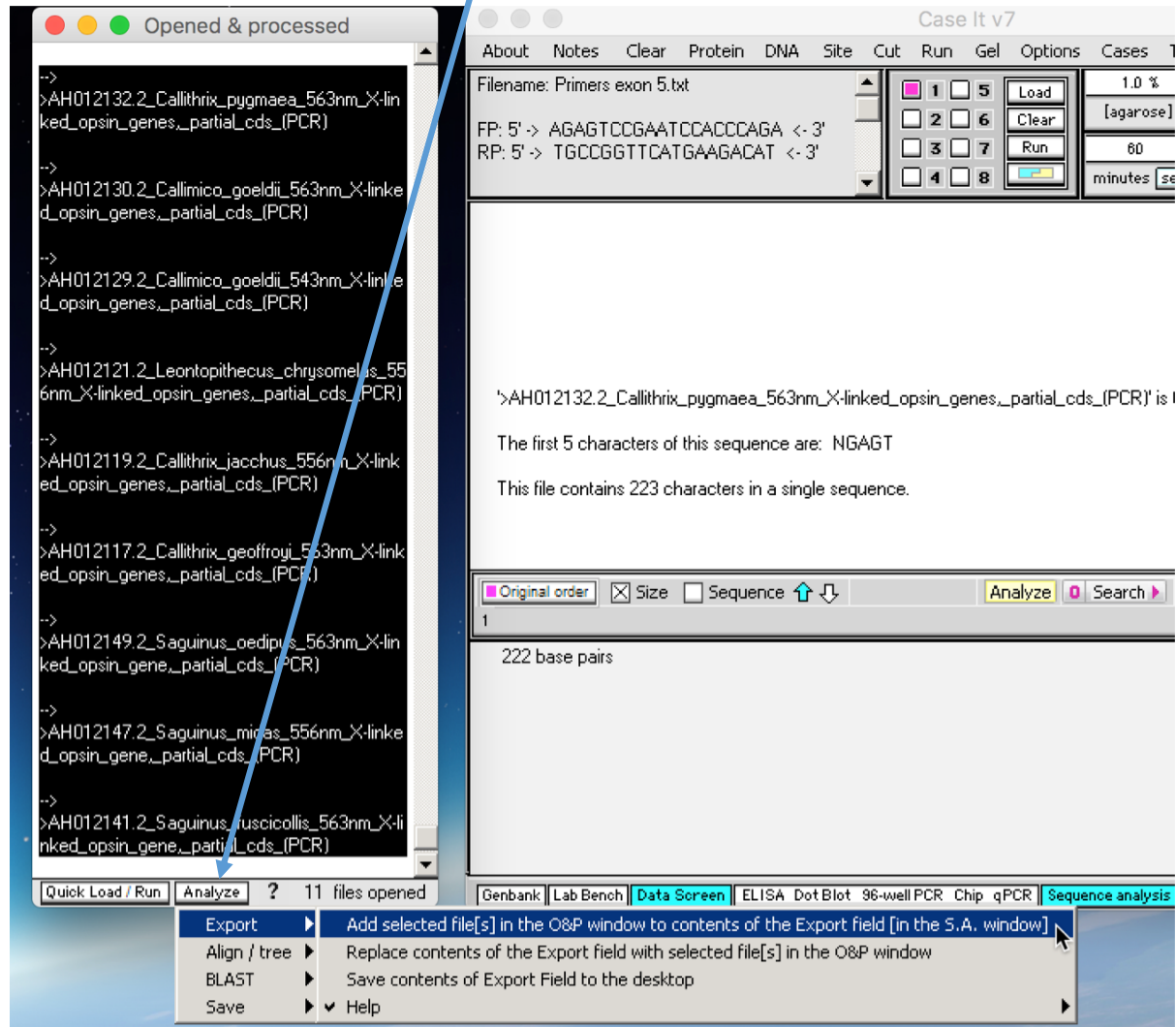


You can experiment with re-running PCR with different match percent settings, to find an optimal setting for particular combinations of primers and sequences being searched during the PCR procedure. Settings that are **too high** may miss FP and/or RP sites, whereas settings that are **too low** may match with spurious FP and RP sites, rendering the results meaningless.

**Note:** The default match % setting is 100% because existing cases were originally developed with primers that matched exactly, and earlier versions of Case It did not have the capability of finding locations with less than a 100% match. With Version 7, it is now possible to use any % match setting, making the simulation more useful for research purposes.

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These PCR products can now be analyzed via multiple alignment and tree-building. First, **shift-click** to highlight all PCR products (lines preceded by arrows) in the **Opened & processed** window, then click the **Analyze** button at the bottom of that window and select **Export -> Add selected file(s)...**



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The PCR products have been added to the Export field of the Sequence Analysis window. Scroll through the Export field to verify that PCR products were generated for all files except >AH012134.2\_Leontopithecus. Select the lines shown highlighted below and delete them by selecting them and hitting the **Delete** key of your keyboard.

The screenshot shows the 'Sequence analysis' window with a list of PCR products. The entry '>AH012121.2\_Leontopithecus' is highlighted in black. A blue arrow points to the right with the word 'Delete' next to it, indicating the action to be taken.

Options Find FASTA

Search results field

TACAACCCATTATCTATGTCTTTATGAACCG  
GCA

>AH012121.2\_Leontopithecus

>AH012119.2\_Callithrix\_jac

NGAGTCCGAATCTACCCAGAAGGCAGAGAAG  
GAAGTGACGCGCATGGTGGTGGTGATGATC  
GCGGCGTACTGTGCTGCTGGGGACCCACA  
CCTTCTTCGCATGCTTTGCTGCTGCCAACCC  
GGTACGCTTCCACCCCTGTGATGGCTGCC  
TGCCAGCCTACTTTGCCAAAAGTGCCACTATC  
TACAACCCATTATCTATGTCTTTATGAACCG  
GCA

>AH012117.2\_Callithrix\_geo

NGAGTCTGAATCCACCCAGAAGGCAGAGAAG  
GAAGTGACGCGCATGGTGGTGGTGATGATC  
GTGGCGTATTGCGTCTGCTGGGGACCCACA  
CCTTCTTCGCATGCTTTGCTGCTGCCAACCC

Enter search sequence

Enter replacement sequence

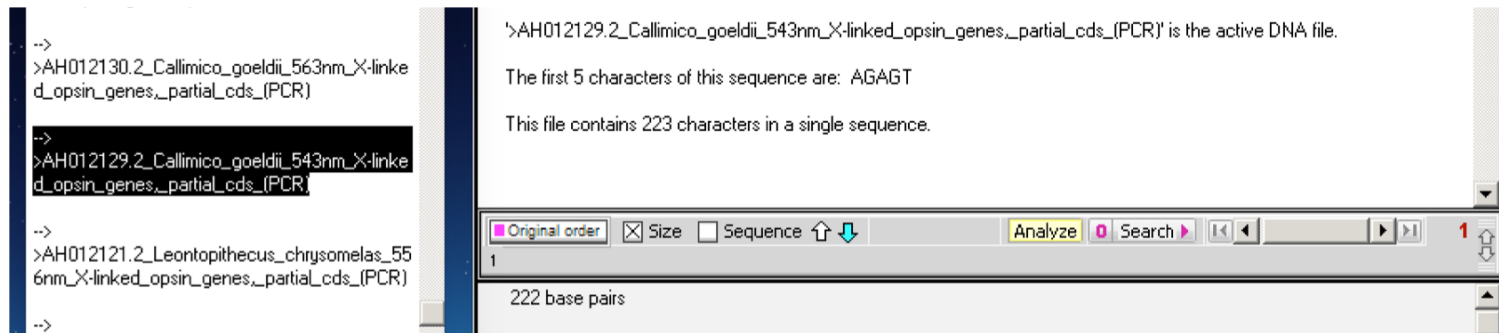
ed Loaded Ge1 / blot Help

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As an aside, it is important to verify that we really have isolated a region associated with exon 5 of the opsin gene. One way to do this is to click on lines in the **Opened & processed** window representing PCR products (lines preceded by arrows), to see if they are all about the same size.

For this example, all PCR products have 222 characters, suggesting that the match percent setting (70%) was about right for isolating exon 5.

Note: If there is a slight discrepancy in the number of characters in the white field above compared to the gray field below (e.g. 223 vs 222), go by the number in the gray field.

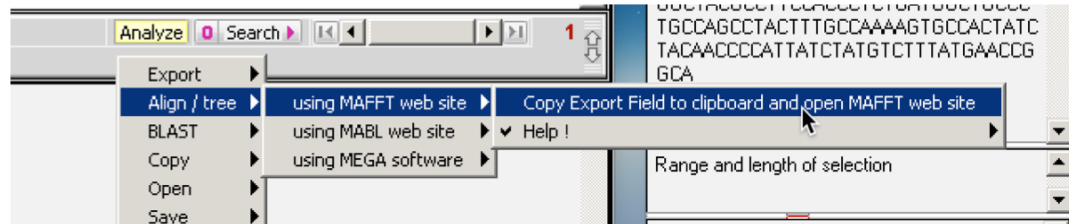


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Previously (slides 24 and 25) we used MEGA software to build a tree, but for this example we'll click on the **Analyze** button and select **Align / tree -> using MAFFT web site -> Copy Export Field to clipboard and open MAFFT web site**.

Note: This particular web site is more reliable than the MABL site, which can be unresponsive at times.



Your default browser will automatically open to the first page of the MAFFT site. **Paste** contents of the clipboard into the Input field.

## MAFFT version 7

Multiple alignment program for amino acid or nucleotide sequences

[Download version](#)

[Mac OS X](#)

[Windows](#)

[Linux](#)

[Source](#)

**Online version**

[Alignment](#)

[mafft --add](#)

[Merge](#)

[Phylogeny](#)

[Rough tree](#)

[Merits / limitations](#)

[Algorithms](#)

[Tips](#)

[Benchmarks](#)

[Feedback](#)

For a large number of short sequences, try [an experimental service](#) (2017/Jul).

### Multiple sequence alignment and NJ / UPGMA phylogeny

**Input:**

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

```
CCAGCCTACTTTGCCAAAAGTGCCACTATCTACAACCCATTATCTATGTCTTTATGAACCGGCA
CCAGCCTACTTTGCCAAAAGTGCCACTATCTACAACCCATTATCTATGTCTTTATGAACCGGCA
CCAGCCTACTTTGCCAAAAGTGCCACTATCTACAACCCGTTATCTATGTCTTTATGAACCGGCA
CCAGCCTACTTTGCCAAAAGTGCCACTATCTACAACCCGTTATCTATGTCTTTATGAACCGGCA
CCAGCCTACTTTGCCAAAAGTGCCACTATCTACAACCCATTATCTATGTCTTTATGAACCGGCA
```

[Back to beginning](#)

Scroll down further on that web page and click the **Submit** button. Note that we will be using the default settings to build the tree.

## MAFFT version 7

Multiple alignment program for amino acid or nucleotide sequences

[Download version](#)

[Mac OS X](#)

[Windows](#)

[Linux](#)

[Source](#)

[Online version](#)

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

Email address:

**Submit**

Reset

On the next page that appears, click **Phylogenetic tree**.

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

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

Refine dataset

**Phylogenetic tree**

On the page that appears after that, click **Go**.

sequence and the  
others.

[Open all plots](#)

NJ or UPGMA tree ( $\beta$ )

sequences,  total sites,  gap-free sites,  [conserved sites](#)

**Go!**

Reset

Finally, click **View tree on Phylo.io** (or one of the other options on that page).

sequence and the  
others.

[Open all plots](#)

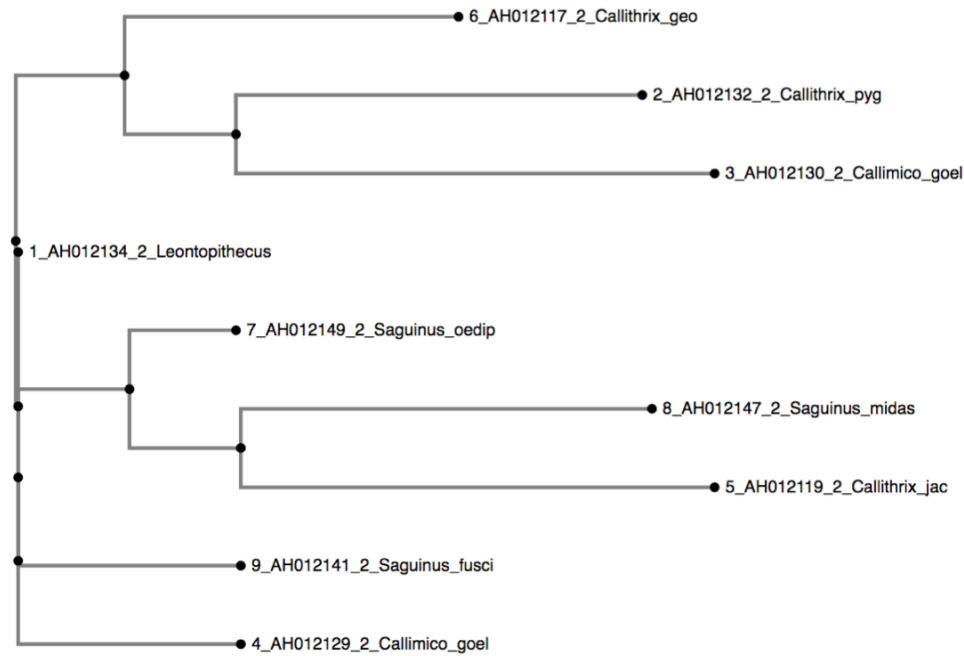
Result (Phylo.io 1.0.0)

Phylo.io runs on any modern browser.

**View tree on Phylo.io**

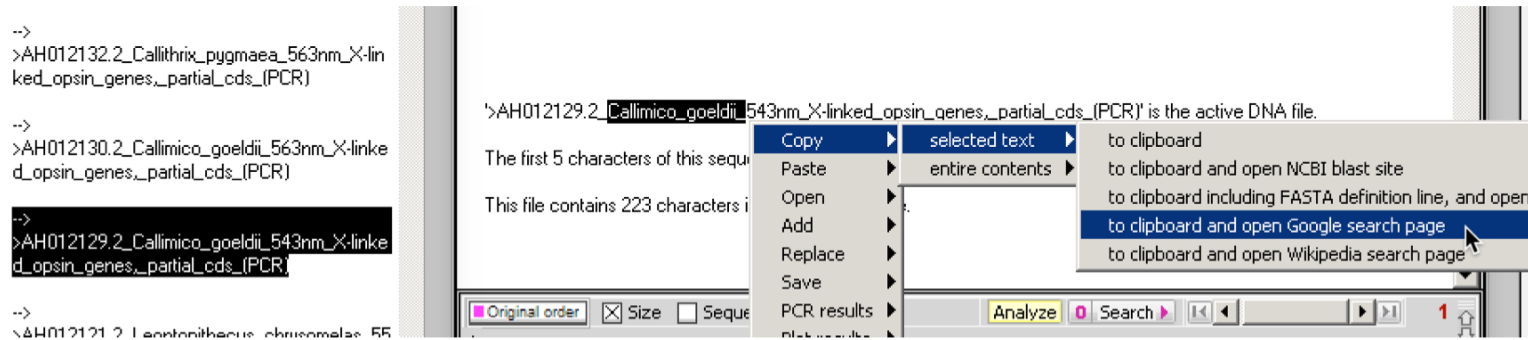
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...and the tree appears. Whether or not this particular tree has any significance depends a number of factors, including whether the default settings were appropriate for this analysis and whether the BLAST results selected were appropriate for the hypothesis being tested. The tree is shown here simply to demonstrate the procedure for tree-building using the MAFFT web site.



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There are other features of Case It v7 that are useful for gathering information for research. For example, you can copy text (such as a scientific name) from the large white field of the Data Screen and automatically open the search page of **Google** or **Wikipedia**, then paste the scientific name and search. This is a quick way to display information about that species, when gathering information for testing hypotheses on ecological, behavioral, evolutionary, or other topics. **This concludes the tutorial.**



### Showing results for *Callimico Goeldii*

Search instead for [Callimico\\_goeldii](#)

#### [Callimico goeldii \(Callimico, Goeldi's Marmoset, Goeldi's Monkey ...](#)

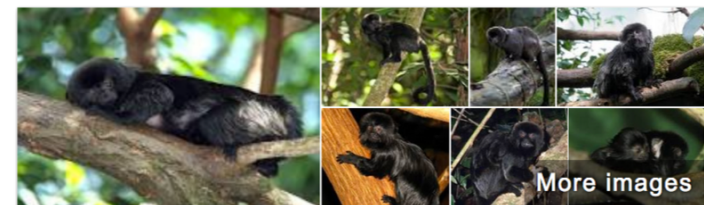
[www.iucnredlist.org/details/3564/0](http://www.iucnredlist.org/details/3564/0) ▼

Range Description: *Callimico goeldii* occurs in the upper Amazon from the Rio Caquetá in Colombia, south through the Peruvian Amazon and the extreme western Amazon of Brazil into the Pando region of northern Bolivia (Hernández-Camacho and Barriga-Bonilla 1966; Hernández-Camacho and Cooper 1976; ...

[Taxonomy](#) · [Assessment Information](#) · [Geographic Range](#) · [Population](#)

#### [Goeldi's marmoset - Wikipedia](#)

[https://en.wikipedia.org/wiki/Goeldi%27s\\_marmoset](https://en.wikipedia.org/wiki/Goeldi%27s_marmoset) ▼



[More images](#)

## Goeldi's marmoset



The Goeldi's marmoset or Goeldi's monkey is a small, South American New World monkey that lives in the upper Amazon basin region of Bolivia, Brazil, Colombia, Ecuador, and Peru. [Wikipedia](#)

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