



INTRODUCTION

Multiple alignments of protein sequences are important tools in studying sequences. The basic information they provide is identification of conserved sequence regions. This is very useful in designing experiments to test and modify the function of specific proteins, in predicting the function and structure of proteins, and in identifying new members of protein families.

Sequences can be aligned across their entire length (global alignment) or only in certain regions (local alignment). This is true for pairwise and multiple alignments. Global alignments need to use gaps (representing insertions/deletions) while local alignments can avoid them, aligning regions between gaps. ClustalW is a fully automatic program for global multiple alignment of DNA and protein sequences. The alignment is progressive and considers the sequence redundancy. Trees can also be calculated from multiple alignments. The program has some adjustable parameters with reasonable defaults.

ClustalW Nucleotide Tutorial

For additional help on ClustalW also see:

- ClustalW FAQ
- ClustalW Improving Sensitivity

YOUR SEQUENCES

Please make sure that your sequences have different names as the first 30 characters of the name are significant. If clustalw finds two or more sequences with the same name it will fail! <u>View example</u>. Click <u>here</u> for more information on sequence errors.

ClustalW currently supports 7 multiple sequence formats. These are:

- NBRF/PIR
- EMBL / UniProtKB/Swiss-Prot
- Pearson (Fasta)
- <u>GDE</u>
- <u>ALN/ClustalW</u>
- GCG/MSF
- <u>RSF</u>

More on Sequence Formats

Please remove any white space space or empty lines from the beginning of your input.

No bootstrap analysis allowed via the website as it is is too cpu intensive .It is possible to perform bootstrap analysis or to get the Bootstrap values along with the nodes of each branch of the phylogram, to do this you will need to download the software (available from the clustal page) and run this locally.

• YOUR EMAIL

You must type your email address in this text box if you are running a job via email. It is not necessary to fill in the box if you are running your search interactively.

• ALIGNMENT TITLE

You may type any text you want to help you identify your search results.

• RESULTS

This option lets you choose between email and interactive runs. The email run requires you to type an email address in the email text box, and your results will be delivered when they are ready to your email address, thus avoiding waiting for your results as with an interactive run. For example: joe@somewhere.domain.country.

The default is interactive.

ALIGNMENT

You may choose to run a full alignment or using a stringent algorithm for generating the tree guide or a fast algorithm.

• CPU MODE

The multiple CPU option run a special version of clustalw using several linux pc nodes in a parallel fashion to increase the speed of the job without compromising the quality of the results. This option is to be chosen when the user has a large number of sequences (50+ but less than 500) to align. However, care should be taken not to overestimate the quantification of the results. A very large alignment is difficult to read and handle by other software.

• OUTPUT

Here you decide which output format you want your multiple sequence alignment in. The options are <u>ALN</u>, <u>GCG</u>, <u>PHYLIP</u>, <u>PIR</u> and <u>GDE</u>. The output results are stored in a ".aln" file which contains your results in <u>ALN</u> format.

You can configure your browser to automatically load the results files from clustalw into an suitable application of your choice. Here is a small list of URL's for obtaining such applications for Win95, Macs and UNIX boxes:

- belvu UNIX Multiple sequence alignment viewer http://www.cgr.ki.se/cgr/groups/sonnhammer/Belvu.html
- njplot UNIX, Mac & PC Tree viewer http://pbil.univ-lyon1.fr/software/njplot.html
- GeneDoc GCG MSF file viewer -(NB - Use GCG format in the OUTPUT option) http://www.psc.edu/biomed/genedoc/
- TreeView Tree viewer for Macs and PC (running Windows)

http://taxonomy.zoology.gla.ac.uk/rod/treeview.html

 ClustalX - Graphical Interface X-Windows/Mac/Win9x based version of ClustalW

ftp://ftp.ebi.ac.uk/pub/software/dos/clustalx/ ftp://ftp.ebi.ac.uk/pub/software/mac/clustalx/ ftp://ftp.ebi.ac.uk/pub/software/unix/clustalx/

- ClustalW Command line version
 <u>ftp://ftp.ebi.ac.uk/pub/software/dos/clustalw/</u>
 <u>ftp://ftp.ebi.ac.uk/pub/software/mac/clustalw/</u>
 <u>ftp://ftp.ebi.ac.uk/pub/software/unix/clustalw/</u>
- CINEMA - A Colour INteractive Editor for Multiple Alignments http://www.bioinf.man.ac.uk/dbbrowser/CINEMA2.1/
- EMMA-An EMBOSS interface to clustalw. http://www.emboss.org/

To configure your browser you must tell it how to handle the following non-registered MIME types:

- application/x-tree njplot %s (or whatever application you choose to handle the MIME type)
- application/x-align belvu %s (or whatever application you choose to handle the MIME type)

<u>JalView</u>: A new experimental option has been added to the results page which involved using a Java Applet called <u>JalView</u>. This is a fully featured MSA editor which allows you not only to edit the alignment but also, to exchange the alignment formats. Please note that JalView is under development. For documentation please click on the <u>JalView</u> Hyperlink or visit the official website at <u>http://www.jalview.org</u>.

• OUTORDER

Decide which order the sequences should be printed in the alignment.

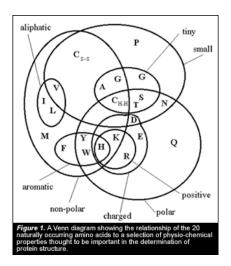
• COLOUR

Show Colors

A button labeled 'Show Colors' will be displayed in the Alignment section of results page. If you press this button the alignment will be show in color according to the table below.

NOTE: NOTE: This option only works when you have chosen ALN or GCG as the output format.

AVFPMILW	RED	Small (small+ hydrophobic (incl.aromatic -Y))
DE	BLUE	Acidic
RHK	MAGENTA	Basic
STYHCNGQ	GREEN	Hydroxyl + Amine + Basic - Q
Others	Gray	



Hide Colors

A button labeled 'Hide Colors' will be displayed in the result page is the 'Show colors' button has been pressed. This option will display the alignment in normal text with no colors.

The default is not to colour the alignments.

CONSENSUS SYMBOLS:

An alignment will display by default the following symbols denoting the degree

of conservation observed in each column:

"*" means that the residues or nucleotides in that column are identical in all

sequences in the alignment.

":" means that conserved substitutions have been observed, according to the COLOUR table above.

"." means that semi-conserved substitutions are observed.

More on sequence/alignment formats

FAST PAIRWISE ALIGNMENT OPTIONS:

• KTUP

This option allows you to choose which 'word-length' to use when calculating fast pairwise alignments.(note: make sure you have chosen 'fast' in the ALIGNMENT.

• WINDOW

Use this option to set the window length when calculating fast pairwise alignments.(Note: make sure you have chosen 'fast' in the

ALIGNMENT.

SCORE

This option allows you to decide which score to take into account when calculating a fast pairwise alignment. (Note: make sure you have chosen 'fast' in the ALIGNMENT.

• TOPDIAG

Select here how many top diagonals should be integrated when calculating a fast pairwise alignment.(Note: make sure you have chosen 'fast' in the ALIGNMENT.

PAIRGAP

Select here to set the gap penalty when generating fast pairwise alignments.

MULTIPLE SEQUENCE ALIGNMENT OPTIONS:

• MATRIX

This option allows you to choose which matrix series to use when generating the multiple sequence alignment. The program goes through the chosen matrix series, spanning the full range of amino acid distances.

- <u>BLOSUM</u> (Henikoff). These matrices appear to be the best available for carrying out data base similarity searches. The matrix used is Blosum30.
- <u>PAM</u> (Dayhoff). These have been extremely widely used since the late '70s. We use the PAM 350 matrix.
- <u>GONNET</u>. These matrices were derived using almost the same procedure as the Dayhoff one (above) but are much more up to date and are based on a far larger data set. They appear to be more sensitive than the Dayhoff series. We use the GONNET 250 matrix.

We also supply an identity matrix which gives a score of 10 to two identical amino acids and a score of zero otherwise.

- Default values are:
 - DNA: DNA Identity matrix.
 - Protein: Gonnet 250.

More on Matrices

• GAPOPEN

You can set here the penalty for opening a gap.

Default values are:

- **DNA:** 15.0
- Protein: 10.0

• ENDGAP

You can set here the penalty for closing a gap.

Default value is -1.

GAPEXT

You can set here the penalty for extending a gap.

Default values are:

- **DNA:** 6.66
- Protein: 0.2

GAPDIST

You can set here the gap separation penalty.

Default value is 4.

More about gaps.

• EXAMPLE

e.g. A multiple sequence alignment was done with ClustalW. 5 sequences were input in the fasta format (<u>Download</u>):

```
>FOSB MOUSE Protein fosB
      MFQAFPGDYD SGSRCSSSPS AESQYLSSVD SFGSPPTAAA SQECAGLGEM PGSFVPTVTA
      ITTSQDLQWL VQPTLISSMA QSQGQPLASQ PPAVDPYDMP GTSYSTPGLS AYSTGGASGS
      GGPSTSTTTS GPVSARPARA RPRRPREETL TPEEEEKRRV RRERNKLAAA KCRNRRELT
      DRLQAETDQL EEEKAELESE IAELQKEKER LEFVLVAHKP GCKIPYEEGP GPGPLAEVRD
      LPGSTSAKED GFGWLLPPPP PPPLPFQSSR DAPPNLTASL FTHSEVQVLG DPFPVVSPSY
      TSSFVLTCPE VSAFAGAQRT SGSEQPSDPL NSPSLLAL
>FOSB HUMAN Protein fosB
      MFQAFPGDYD SGSRCSSSPS AESQYLSSVD SFGSPPTAAA SQECAGLGEM PGSFVPTVTA
      ITTSQDLQWL VQPTLISSMA QSQGQPLASQ PPVVDPYDMP GTSYSTPGMS GYSSGGASGS
      GGPSTSGTTS GPGPARPARA RPRRPREETL TPEEEEKRRV RRERNKLAAA KCRNRRELT
      DRLQAETDQL EEEKAELESE IAELQKEKER LEFVLVAHKP GCKIPYEEGP GPGPLAEVRD
      LPGSAPAKED GFSWLLPPPP PPPLPFQTSQ DAPPNLTASL FTHSEVQVLG DPFPVVNPSY
      TSSFVLTCPE VSAFAGAQRT SGSDQPSDPL NSPSLLAL
>FOS CHICK Proto-oncogene protein c-fos
      MMYQGFAGEY EAPSSRCSSA SPAGDSLTYY PSPADSFSSM GSPVNSQDFC TDLAVSSANF
      VPTVTAISTS PDLQWLVQPT LISSVAPSQN RGHPYGVPAP APPAAYSRPA VLKAPGGRGQ
      SIGRRGKVEQ LSPEEEEKRR IRRERNKMAA AKCRNRRREL TDTLQAETDQ LEEEKSALQA
      EIANLLKEKE KLEFILAAHR PACKMPEELR FSEELAAATA LDLGAPSPAA AEEAFALPIM
      TEAPPAVPPK EPSGSGLELK AEPFDELLFS AGPREASRSV PDMDLPGASS FYASDWEPLG
AGSGGELEPL CTPVVTCTPC PSTYTSTFVF TYPEADAFPS CAAAHRKGSS SNEPSSDSLS
      SPTLLAL
>FOS_RAT Proto-oncogene protein c-fos
      MMFSGFNADY EASSSRCSSA SPAGDSLSYY HSPADSFSSM GSPVNTQDFC ADLSVSSANF
IPTVTAISTS PDLQWLVQPT LVSSVAPSQT RAPHPYGLPT PSTGAYARAG VVKTMSGGRA
      QSIGRRGKVE QLSPEEEEKR RIRRERNKMA AAKCRNRRRE LTDTLQAETD QLEDEKSALQ
      TEIANLLKEK EKLEFILAAH RPACKIPNDL GFPEEMSVTS LDLTGGLPEA TTPESEEAFT
      LPLLNDPEPK PSLEPVKNIS NMELKAEPFD DFLFPASSRP SGSETARSVP DVDLSGSFYA
ADWEPLHSSS LGMGPMVTEL EPLCTPVVTC TPSCTTYTSS FVFTYPEADS FPSCAAAHRK
      GSSSNEPSSD SLSSPTLLAL
>FOS_MOUSE Proto-oncogene protein c-fos
      MMFSGFNADY EASSSRCSSA SPAGDSLSYY HSPADSFSSM GSPVNTQDFC ADLSVSSANF
      IPTVTAISTS PDLQWLVQPT LVSSVAPSQT RAPHPYGLPT QSAGAYARAG MVKTVSGGRA
      QSIGRRGKVE QLSPEEEEKR RIRRERNKMA AAKCRNRRRE LTDTLQAETD QLEDEKSALQ
      TEIANLLKEK EKLEFILAAH RPACKIPDDL GFPEEMSVAS LDLTGGLPEA STPESEEAFT
      LPLLNDPEPK PSLEPVKSIS NVELKAEPFD DFLFPASSRP SGSETSRSVP DVDLSGSFYA
      ADWEPLHSNS LGMGPMVTEL EPLCTPVVTC TPGCTTYTSS FVFTYPEADS FPSCAAAHRK
      GSSSNEPSSD SLSSPTLLAL
```

Output was in the format:

The sequences are aligned with each other, with the query sequence at the top and subsequent sequences below. Gaps are represented by the "-" symbol. The running total number of amino acids or nucleotides are shown on the right. The degree of similarity is illustrated underneath the alignments with a series of consensus symbols.

Consensus Symbols

An alignment will display by default the following symbols denoting the degree of conservation observed in each column:

"*" means that the residues or nucleotides in that column are identical in all sequences in the alignment.

":" means that conserved substitutions have been observed, according to the COLOUR table <u>above.</u>

"." means that semi-conserved substitutions are observed.

FOS_RAT FOS_MOUSE FOS_CHICK FOSB_MOUSE FOSB_HUMAN	MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANF MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANF MMYQGFAGEYEAPSSRCSSASPAGDSLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANF -MFQAFPGDYDS-GSRCSS-SPSAESQYLSSVDSFGSPPTAAASQE-CAGLGEMPGSF -MFQAFPGDYDS-GSRCSS-SPSAESQYLSSVDSFGSPPTAAASQE-CAGLGEMPGSF *:* .:*:: .***** **:::* * ***** : :*: *:.*	60 60 54 54
FOS_RAT FOS_MOUSE FOS_CHICK FOSB_MOUSE FOSB_HUMAN	IPTVTAISTSPDLQWLVQPTLVSSVAPSQTRAPHPYGLPTPS-TGAYARAGVV IPTVTAISTSPDLQWLVQPTLVSSVAPSQTRAPHPYGLPTQS-AGAYARAGMV VPTVTAISTSPDLQWLVQPTLISSVAPSQNRG-HPYGVPAPAPPAAYSRPAVL VPTVTAITTSQDLQWLVQPTLISSMAQSQGQPLASQPPAVDPYDMPGTSYSTPGLS VPTVTAITTSQDLQWLVQPTLISSMAQSQGQPLASQPPVVDPYDMPGTSYSTPGMS :******:** ***************************	112 112 112 110 110
FOS_RAT FOS_MOUSE FOS_CHICK FOSB_MOUSE FOSB_HUMAN	KTMSGGRAQSIGRRGKVEQLSPEEEEKRRIRRERNKMAAA KTVSGGRAQSIGRRGKVEQLSPEEEEKRRIRRERNKMAAA KAP-GGRGQSIGRRGKVEQLSPEEEEKRRIRRERNKMAAA AYSTGGASGSGGPSTSTTTSGPVSARPARARPRRPREETLTPEEEEKRRVRRERNKLAAA GYSSGGASGSGGPSTSGTTSGPGPARPARARPRRPREETLTPEEEEKRRVRRERNKLAAA :** . * *.::: :::: .: ** : * *:*******	152 151 170
FOS_RAT FOS_MOUSE FOS_CHICK FOSB_MOUSE FOSB_HUMAN	KCRNRRELTDTLQAETDQLEDEKSALQTEIANLLKEKEKLEFILAAHRPACKIPNDLGF KCRNRRELTDTLQAETDQLEDEKSALQTEIANLLKEKEKLEFILAAHRPACKIPDDLGF KCRNRRELTDTLQAETDQLEEEKSALQAEIANLLKEKEKLEFILAAHRPACKMPEELRF KCRNRRELTDRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEG- KCRNRRELTDRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEG- ********** **************************	212 212 211 229 229
FOS_RAT FOS_MOUSE FOS_CHICK FOSB_MOUSE FOSB_HUMAN	PEEMSVTS-LDLTGGLPEATTPESEEAFTLPLLNDPEPK-PSLEPVKNISNMELKAEPFD PEEMSVAS-LDLTGGLPEASTPESEEAFTLPLLNDPEPK-PSLEPVKSISNVELKAEPFD SEELAAATALDLGAPSPAAAEEAFALPLMTEAPPAVPPKEPSGSGLELKAEPFD PGPGPLAEVRDLPGSTSAKEDGFGWLLPPPPPPPLPFQ PGPGPLAEVRDLPGSAPAKEDGFSWLLPPPPPPPLPFQ : ** . : *:.* * . *	270 270 265 267 267
FOS_RAT FOS_MOUSE FOS_CHICK FOSB_MOUSE FOSB_HUMAN	DFLFPASSRPSGSETARSVPDVDLSGSFYAADWEPLHSSSLGMGPMVTELEPLCTPVV DFLFPASSRPSGSETSRSVPDVDLSGSFYAADWEPLHSNSLGMGPMVTELEPLCTPVV ELLFSAGPREASRSVPDMDLPGASSFYASDWEPLGAGSGGELEPLCTPVV SSRDAP-PNLTA-SLFTHSEVQVLGDPFP TSQDAP-PNLTA-SLFTHSEVQVLGDPFP :::* :* *::: . *:: *	328 328 315 294 294
FOS_RAT FOS_MOUSE FOS_CHICK FOSB_MOUSE FOSB_HUMAN	TCTPSCTTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 380 TCTPGCTTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 380 TCTPCPSTYTSTFVFTYPEADAFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 367 VVSPSYTSSFVLTCPEVSAFAGAQRTSGSEQPSDPLNSPSLLAL 338 VVNPSYTSSFVLTCPEVSAFAGAQRTSGSDQPSDPLNSPSLLAL 338 * :***:**:* **:* *.*:* :*.:: .**.****	

• EDITING AN ALIGNMENT

You can edit the alignment using <u>jalview</u>. Click on the button below to view the above alignment.

• PHYLOGENETIC TREE

Phylogram is a branching diagram (tree) assumed to be

an estimate of a phylogeny, branch lengths are proportional to the amount of inferred evolutionary change. A Cladogram is a branching diagram (tree) assumed to be an estimate of a phylogeny where the branches are of equal length, thus cladograms show common ancestry, but do not indicate the amount of evolutionary "time" separating taxa. Tree distances can be shown, just click on the diagram to get a menu of options. The ".dnd" file is a file that describes the phylogenetic tree.

These are now in controlled with new buttons in the output file as well as a pop up menu, that is available by right-clicking on the applet. The buttons on the page include "Show as Phylogram Tree", "Show as Cladogram Tree" and "Show Distances".

IMPORTANT!

Please note applets are not printed out with html pages, You will need to:

- Use the "Print Screen" button in the top right corner of your keyboard.
- Open an imaging application like paint or photoshop.
- Go "file>new" from the menu or "control+N" from the keyboard to create a new image.
- Go "edit>paste from the menu or "control+V" from the keyboard to paste your screen capture.
- The use the crop function to trim the image (e.g. "image>crop").
- Then save or print the image.

If you cannot see the tree, your java plug in is probably not working, please reinstall java on your machine or enable it in your internet settings. You can download java from <u>http://java.sun.com/</u>. If you have java running, you can see the version of java you are running in the box below (applet from <u>http://www.javatester.org</u>). You should have at least java version 1.2 to run this applet.

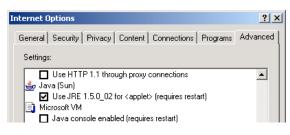
The vendor and version from the JVM:

You should see a pink box above with one line of text that says something like:

Java Version: 1.1.4 from Microsoft Corp. or Java Version 1.4.2_05 from Sun Microsystems Inc. or Java Version: 1.3.1 from Apple Computer, Inc. or Java Version: 1.1.5 from Netscape Communications Corporation

For actual results, see expected output.

Example of enabling Java in Internet Explorer (Windows)

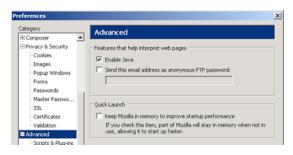


Examples of Enabling Java in Firefox (Windows/Linux)





Example of Enabling java in Mozilla (Windows)



Example of Enabling java in Safari (Mac)



example:

Right-click in the area below for options!

IMPORTANT:

To use this option you will need to input a sequence alignment. Please make sure this alignment is in PIR or PHYLIP format. ALN and GCG MSF files are not supported so you will have to convert your MSF files to PIR format with, for example, GCG's ToPir:

topir pileup.msf{*} -outf=pileup.pir

Please refer to the GCG documentation to find out how to use this program correctly. You may then use this file as input (cut and paste or upload) to this service.

The method used is the NJ (Neighbour Joining) method of Saitou and Nei. First you calculate distances (percent divergence) between all pairs of sequence from a multiple alignment; second you apply the NJ method to the distance matrix.

This option allows you to choose the following output formats for the tree:

- Neighbour
- Phylip
- Distance

In order to view these trees you must have a program capable of displaying the data. Please refer to this pages section on <u>OUTPUT</u> for more information.

• Kimura Correction of distances

This options allows you to set on distances correction (correction for multiple substitutions). This is because, as sequences diverge, more than one substitution will happen at many sites. However, you only see one difference when you look at the present day sequences. Therefore, this option has the effect of stretching branch lengths in trees (especially long branches). The corrections used here (for DNA or proteins) are both due to Motoo Kimura.

• Ignore Gaps in alignment

With this option, any alignment positions where ANY of the sequences have a gap will be ignored. This means that 'like' will be compared to 'like' in all distances. It also, automatically throws away the most ambiguous parts of the alignment, which are concentrated around gaps (usually). The disadvantage is that you may throw away much of the data if there are many gaps.

• UPLOAD A FILE

You may upload a file from your computer which containing a valid **set** of sequences in any format (<u>GCG, FASTA, EMBL, GenBank, PIR, NBRF, Phylip</u> or <u>UniProtKB/Swiss-Prot</u>) using this option. Please note that this option only works with Netscape Browsers or Internet Explorer version 5 or later. Some word processors may yield unpredictable results as hidden/control characters may be present in the files. It is best to save files with the Unix format option to avoid hidden windows characters. Some examples of common sequence formats may be seen <u>here</u>.

• SCORES TABLE

Scores Table is a new view to ClustalW output. Users can sort the scores by Alignment Score, Sequence Number, Sequence Name and Sequence Length.

• REFERENCES

Higgins D., Thompson J., Gibson T. Thompson J. D., Higgins D. G., Gibson T. J.(1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

ClustalW WWW Service at the European Bioinformatics Institute <u>http://www.ebi.ac.uk/clustalw</u> Rodrigo Lopez, Services Program

Andrew Lloyd The Clustal W WW server at the EBI embnet.news volume 4.2 1997 http://www.ebi.ac.uk/embnet.news/vol4 3/clustalw1.html

The program in use in this service can be obtained freely from: http://www.sgi.com/chembio/resources/clustalw/parallel_clustalw.html

Parry-Smith, D.J., Payne, A.WR, Michie, A.D. and Attwood, T. K. (1997) CINEMA - A novel Colour INteractive Editor for Multiple Alignments. *Gene, 211(2), GC45-56.* [Over http://www.bioinf.man.ac.uk/dbbrowser/CINEMA2.1/

Attwood, T. K., Payne, A. W.R., Michie, A.D. and Parry-Smith, D.J. (1997) A Colour INteractive Editor for Multiple Alignments - CINEMA, <u>EMBnet.news, 3 (3).</u>

Jalview - a java multiple alignment editor

http://www.jalview.org

pfaat (Protein Family Alignment Annotation Tool) http://pfaat.sourceforge.net/

• OTHER SERVICES:

This services is also available as an application from the EBI's srs server: <u>http://srs.ebi.ac.uk/</u>

N.B. <u>DbClustal</u> can be launched from <u>Wu-Blast2</u> and <u>NCBI-Blast2</u> you can choose some of the sequences from your score list and align them with your query sequence. You may also then paste your .aln files back into ClustalW (bootstrapping) in order to <u>colour</u> your alignments, view the <u>phylogenetic trees</u> or launch <u>jalview</u>.