Protein Engineering vol.6 no.1 pp.37–40, 1993

ALSCRIPT: a tool to format multiple sequence alignments

Geoffrey J.Barton

University of Oxford, Laboratory of Molecular Biophysics, The Rex Richards Building, South Parks Road, Oxford OX1 3QU, UK

Introduction

Alignments of protein and nucleic acid sequences are a central aid to understanding biological systems. Although several effective tools now exist for the rapid automatic alignment of large numbers of sequences (see, for example, Barton and Sternberg, 1987; Feng and Doolittle, 1987; Higgins and Sharp, 1989; Vingron and Argos, 1989), the preparation for publication of quality figures of such alignments with annotations is often extremely difficult.

Artwork using pen/ink/dry lettering can allow labelling and boxing. However, this involves a great deal of labour and a fair degree of skill to produce visually pleasing results [e.g. the large 128 sequence globin alignment in Barton and Sternberg (1987)]. With the advent of powerful word-processing and graphics packages for personal computers, many laboratories 'tidy up' their alignments by judicious mouse-work. Although good results may be obtained by this route, the process, in common with conventional artwork, is rather inflexible. A particular problem is that having chosen a number of characters per line and character point size, it is difficult subsequently to modify the figure. It is also laborious to add new sequences to an existing figure. In addition, one is normally limited to the use of one of a few fixed-width fonts (e.g. Courier) rather than the full range of fonts available to the word-processor. The table-drawing facilities of various packages can overcome the problems of proportional fonts. For example, the Unix 'troff' macros 'tbl' were used to prepare the alignments in Barton and Sternberg (1990), though subsequently retyped by the *J. Mol. Biol.*! LaTeX (Lamport, 1986) tables were used for the 88 sequence annexin alignment in Barton *et al.* (1991) and the 67 sequence SH2 domain alignment in Russell *et al.* (1992); both figures were extremely time consuming to prepare, but rather easier to update than a conventionally word-processed alignment.

The ALSCRIPT program described in this article was developed specifically to allow the easy formatting and graphical display of large multiple sequence alignments. Although written originally for the author's use, the interface is relatively friendly, and should be easy to learn by anyone familiar with plotting graphs.

Description of ALSCRIPT

ALSCRIPT takes a multiple sequence alignment in the simple AMPS (Barton and Sternberg, 1987; Barton, 1990) block-file format and a set of formatting commands, and produces a PostScript file that may be printed on a PostScript laser printer or viewed using a PostScript previewer (e.g. Sun Microsystem's PageView program). GCG 'MSF' format files and CLUSTAL format files (PIR) are also supported. ALSCRIPT is strictly a formatting, display and annotation tool. It is not a program for multiple sequence alignment, or editing. The previous programs that offer the closest functionality to ALSCRIPT are PRETTYPLOT and PRETTYBOX as supplied with the GCG package (Devereux *et al.*, 1984), and the latest colour version of SOMAP (Parry-Smith and Attwood, 1991). Whilst these programs do not provide the same degree of flexibility in display as offered by ALSCRIPT, they do allow the calculation of consensus sequences and automatic shading/boxing according to defined rules. The aim of ALSCRIPT is to allow the user total control over the display of the sequence alignment; such control is essential if non-sequence information is to be used to highlight features of the sequence: for example, the location of active-site residues, the positions of secondary structures (α-helices and β-strands), and domain or intron/exon boundaries. The flexibility of ALSCRIPT also permits it to be used as a 'front-end' display tool for programs that offer sophisticated alignment analysis features. For example, the AMAS (Analysis of Multiply Aligned Sequences; C.D.Livingstone and G.J.Barton, in preparation) system, which highlights structurally important regions of a multiple alignment, generates ALSCRIPT commands as one output option. Similarly, the consensus algorithms encoded in the GCG program PRETTY could be used to generate ALSCRIPT shading, boxing or font-changing commands for graphical output.

Given a block-file and the text point size, ALSCRIPT calculates how many residues can be fitted across the page and how many sequences will fit down it. It then prints the alignment at the chosen point size on as many pages as are needed. Running ALSCRIPT with a smaller or larger point size will automatically rescale the alignment to fit on fewer or more pages, as appropriate. The actual page dimensions may be reset to any value, so if an A3 PostScript printer or typesetting machine is available, alignments can readily be scaled to make best use of the extra space.

Each output page has three regions. The left hand edge contains identifying text for each sequence, the main part of the page holds the alignment, and the top part, the position numbers and optional tick marks. ALSCRIPT commands make use of a character coordinate system for font changes and other formatting commands. Thus, any residue in the alignment may be referred to by its sequence position number (x-axis) and sequence number (y-axis). Ranges of residue positions or sequences may also be defined in the character coordinate system.

The basic ALSCRIPT commands allow the following functionalities.

Fonts

Any PostScript font in any size may be defined and used on individual residues, regions or identifier codes.

Boxing

Simple rectangular boxes may be drawn around any part of the alignment. Particular residue types may be selected and automatically 'surrounded' by lines. For example, if the characters 'G' and 'P' are selected, lines will not be drawn

a

```
FFFLLFFFFFFFFFFFFLLDDDDDDDDDDDDDDDDDD    HHHHHHH         E       TTTTH
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAVVAAAAAAA7  HHHHHHHH                 TTTH
EEEEEEDDDEEEEEDDEEEGRRRRRRQQQQQQQQQQQQ   HHHHHHHH                  TTH
KKKTTTRRRRRKRRKKRRRREDDAAAVAADDDDDDITR   HHHHHHHHH        E        TTTH
LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL+  HHHHHHHHH         E        TTTH
HYYYYYYYYYYYFFYYYYHHYYYYYYFFFYYYYYYYYY   HHHHHHHHH         E         TTH
QEEYYYDDDKKKKKKKYYRQDDDEEERQQEEEEEAKDQ   HHHHHHHHH         E         TTH
AAAAASSSSSSSAASSAAAAAAAAAAAAAAAAAAAAA7   HHHHHHHH          E         TTH
                      GGGGGGGGGGGGGGGGGG          H                   T
MMMMMMMMMMMMMMMMMLLVVVEEEEEEEEEEEEEE    HHHHHHHH         E        TTTH
KKKKKKKKKKKKKKKKKKKKKKRRRLLLLLKKKKNKG    HHHHHHH         E       TTTTH
GGGGGGGGGGGGGGGGGGGRRRRRRKKKKKKKKIRKR       HHH                TTTTT
VAAAAAKKKLLLLLAAAAIAKKKKKKWWWWWWWWRWWL      HHH                TTTTT
GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG+      HH               TTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT+      HHHH             TTTTTTTTTT
RRRDDDRRRDDDRRDDRDDDDDDDDDDDDDDDDDDDDD      HHHHH            TTTTTTTTTT
HHHDDDDDDDDDDDEEDDEEVVVVVVVEEEEEEEEEEE    HHHHHHHH         E TTTTTTTTTT
KKKHHDKKKNDNNNKKGSFFPPPNNNEEEAAVVVMDDS     HHHHHHH         E   TTTTTTTY
AATTTTVVVTTTTTTTTTTTKKKVVVTKKQQKKKKKKC    HHHHHHHH       EEE      TTTTTH
LLLLLLLLLLLLLLLLLLLLWWWFFFFFFFFFFFFFF6    HHHHHHH       EEEE        TTH
IIIIIIIIIIIIIIITTIVNNIIINTNIIIIILLLITTN5   HHHHHHH       EEEE         TH
RRRRRRRRRRRRRRRRRRRSSSTTTTTTYYTTTTEEM      HHHHHHH      EEEEE          H
IIIVVVIIIVVVIIIVNIIIIIIIIIIIIIIIVVVIIII5    HHHHHH      EEEEE          H
MMMIMMMMMMMMMMMMIVMMMMMLLLLFLLLLLLLLL6     HHHHHH      EEEEE         TH
VVVVVVVVVVVVVVVVVVVVTTTTTTGGGGGCCCCCCA6     HHHHH       EEEE       TTTTH
SSSSSSSSSSSSSSSSTSSEEETSTTTTNNSSSTLLT       HHHH        EE       TTTTTT
RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR+      HHHH        EE  TTTTTTTTTT
```

b

 # (A) Input and output files:

```
BLOCK_FILE     example1.bloc
OUTPUT_FILE    example1.ps
```

 # (B) Page Layout and overall spacing:

```
ADD_SEQ  38  1
ADD_SEQ  39  3
ADD_SEQ  69  1
PORTRAIT
POINTSIZE   10
```

 # (C) Font definitions:

```
DEFINE_FONT 0 Helvetica       DEFAULT
DEFINE_FONT 1 Helvetica REL   0.75
DEFINE_FONT 3 Helvetica-Bold DEFAULT
DEFINE_FONT 4 Times-Bold      DEFAULT
DEFINE_FONT 5 Helvetica-BoldOblique  DEFAULT
DEFINE_FONT 6 Times-Roman DEFAULT
SETUP
```

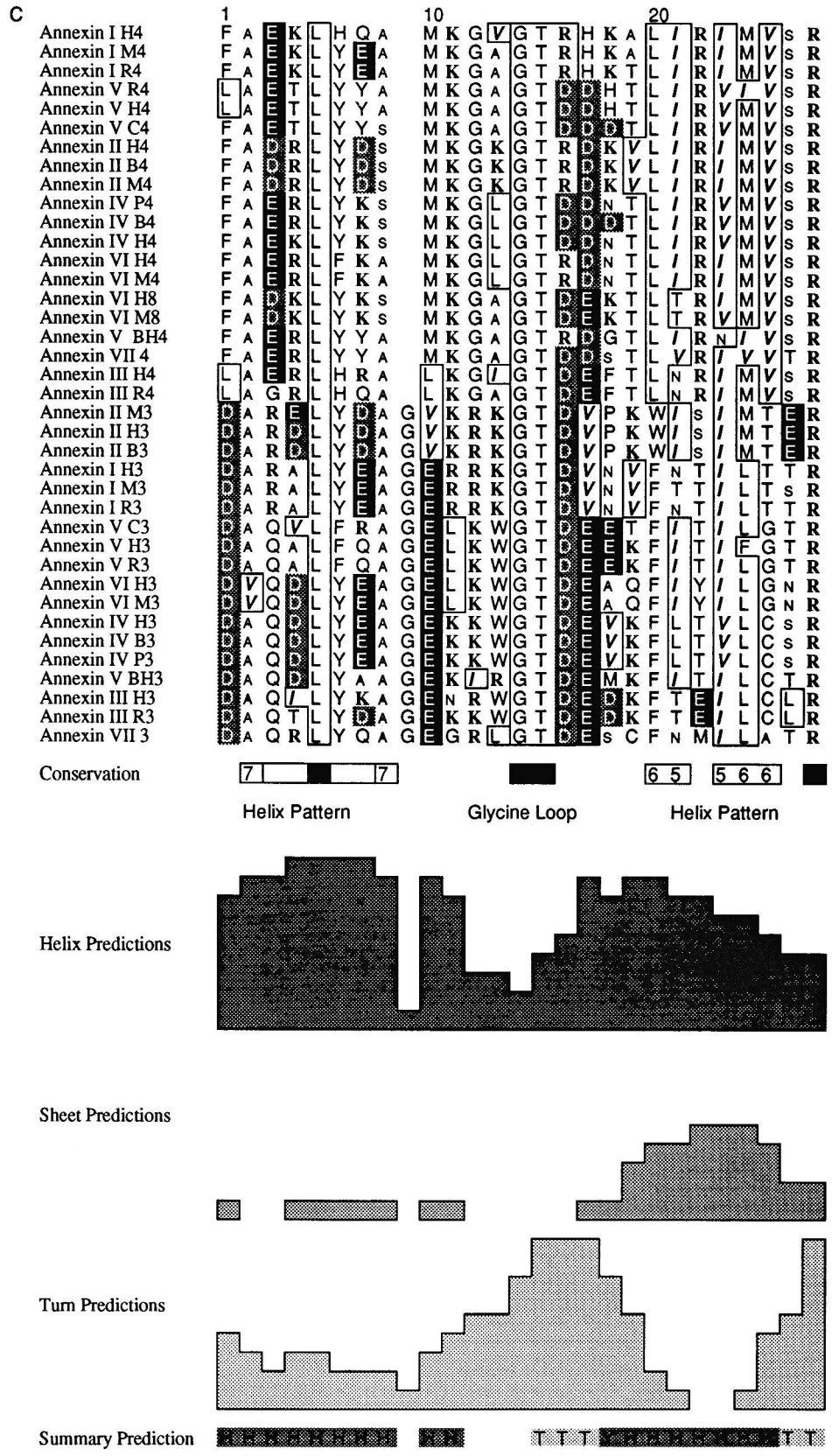
 # (D) Basic formatting and annotation commands:

```
IDENT_FONT ALL 6
SURROUND_CHARS LIV   ALL
FONT_CHARS      IV    ALL  5
FONT_CHARS      ASN   ALL  1
FONT_CHARS      KR    ALL  4
BOX_REGION      14  1  16 38
SHADE_CHARS      +    ALL   0.0
INVERSE_CHARS DE ALL
SHADE_CHARS D ALL 0.5
SHADE_CHARS E ALL 0.0
SURROUND_CHARS 56+789  1 40 27 40
LINE      TOP     2     40     8
LINE   BOTTOM     2     40     8
TEXT   2      42  "Helix Pattern"
TEXT   12     42  "Glycine Loop"
TEXT   21     42  "Helix Pattern"
SUB_ID        49  "Helix Predictions"
SUB_ID        58  "Sheet Predictions"
SUB_ID        68  "Turn Predictions"
```

 # (E) Now improve the appearance of the prediction histograms.

```
SURROUND_CHARS  H     1     44     27     53
SHADE_CHARS     H     1     44     27     53      0.5
SUB_CHARS       1    44     27     53   H SPACE
```

Fig. 1. Illustration of some of the formatting capabilities of ALSCRIPT. (**a**) A small AMPS block file. Each aligned sequence is represented by a column. This block file includes character-based histograms that show the result of a combined secondary structure prediction method (Russell *et al.*, 1992). (**b**) An ALSCRIPT command file. Comment lines start with a # character, sections A−C set up general layout definitions: the ADD_SEQ command allows extra space to be inserted in the alignment at any location; 39 3 means add space for three sequences after sequence 39. PORTRAIT specifies that the output will be arranged with the longest side of the paper vertical. POINT SIZE sets the default point size for plotting characters. The DEFINE_FONT commands set up the fonts that are to be used and their point size; font 0 is used as the default. The point size may be set to the default value, an explicit value or relative (REL) to the default size. Sections D and E show the boxing, shading and font changing commands in action. The IDENT_FONT ALL 6 specifies font 6 (Times-Roman) for all identifiers. SURROUND_CHARS LIV ALL specifies that all L, I and V characters will be separated from other characters by a line (e.g. at sequence positions 19−21). FONT_CHARS KR ALL 4 switches the font of K and R characters to font 4 (Times-Bold 10 point). When regions are selected the coordinates are given in character units with the origin at the top left hand corner of the plotted alignment. For example, BOX_REGION 14 1 16 38 means box from residue 14 to residue 16 of sequences 1−38. These coordinates apply irrespective of how many pages are needed to plot the alignment at the chosen point size. The SHADE_CHARS command permits specific characters to be shaded with a grey value; The INVERSE_CHARS command allows white lettering to be superimposed on the shaded background, as shown for D and E. LINE TOP 2 40 8 draws a horizontal line at the top of the characters from residue 2 to residue 8 of sequence 40, and similar commands allow lines at the left, right and below the defined residues; SUB_ID changes the identifier code for a sequence and SUB_CHARS allows a character to be substituted with another. In the example shown here (**c**), SUB_CHARS is used to remove the H, E and T characters that made up the prediction histograms and replace them with SPACE ' ' characters. The similar commands used to format the Sheet, Turn and Summary predictions are omitted for brevity.



between G and P characters, but only where G and P border with other characters.

Shading

Grey shading of any level from black to white may be applied to any region of the alignment, either as a rectangular region or as residue-specific shading, e.g. to 'shade all Cys residues between positions 6 and 30'.

Text

Specific text strings may be added to the alignment at any position and in any font or font size.

Lines

Horizontal or vertical lines may be drawn to the left, right, top or bottom of any residue position or group of positions.

Defaults

All defaults, e.g maximum number/length of sequences, character spacing, may be modified using ALSCRIPT commands without the need to recompile the program.

Figure 1(a) illustrates a small section of a multiple sequence alignment in AMPS block-file format. The sequence identifier codes stored above the alignment have been deleted for brevity. In this example, the block-file also contains character-based histograms representing the prediction of helix, strand and turn. Figure 1(c) illustrates the result of running ALSCRIPT on this file using the commands shown in Figure 1(b). It is not suggested that this combination of options gives the clearest representation of the data; rather, the options have been chosen to illustrate many of the capabilities of the program.

Although written with the aim of producing figures for publication, ALSCRIPT is a useful research tool for interpreting multiple sequence alignments. For example, the boxing, shading and font changing facilities can be applied to highlight amino acids of a particular type and thus draw attention to clusters of positive or negative charge, hydrophobicity and so on. Furthermore, computer programs for the automatic analysis of alignments can be made to produce ALSCRIPT formatting commands and a block file, thus simplifying the task of generating graphical representations of such analyses.

System requirements and availability

ALSCRIPT is written in C and should compile and run on most computers with a C compiler. An IBM-compatible disk (1.44 MB) including the source code and a compiled version of the program for 386 DOS computers is available from the author.

Acknowledgements

The author thanks the Royal Society for support, and Professor L.N.Johnson for providing a stimulating working environment.

References

Barton,G.J. (1990) *Methods Enzymol.*, **183**, 403–428.
Barton,G.J. and Sternberg,M.J.E. (1987) *J. Mol. Biol.*, **198**, 327–337.
Barton,G.J. and Sternberg,M.J.E. (1990) *J. Mol. Biol.*, **212**, 389–402.
Barton,G.J., Newman,R.H., Freemont,P.F. and Crumpton,M.J. (1991) *Eur. J. Biochem.*, **198**, 749–760.
Devereux,J., Haeberli,P. and Smithies,O. (1984) *Nucleic Acids Res.*, **12**, 387–395.
Feng,D.F. and Doolittle,R.F. (1987) *J. Mol. Evol.*, **25**, 351–360.
Higgins,D.G. and Sharp,P.M. (1989) *Comput. Appl. Biosci.*, **5**, 151–153.
Lamport,L. (1986) *LaTeX: A Document Preparation System*. Addison-Wesley, New York.
Parry-Smith,D.J. and Attwood,T.K. (1991) *Comput. Appl. Biosci.*, **7**, 233–235.
Russell,R.B., Breed,J. and Barton,G.J. (1992) *FEBS Lett.*, **304**, 15–20.
Vingron,M. and Argos,P. (1989) *Comput. Appl. Biosci.*, **5**, 115–121.

Received on June 30, 1992; revised on October 14, 1992; accepted on October 20, 1992