MCB 371/372

Sequence alignment

Sequence space

4/4/05

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Questions on the Needlemann Wunsch Algorithm?

a step by step illustration is here
a more realistic example is here

a) **fill in scoring matrix**
b) **calculate max. possible score for each field**
c) **trace back alignment through matrix**

assignment:

On Monday we used the perl script extract_lines.pl .

Modify the script so that it prints out an additional column that for each blasthit gives the bitscore of the alignment divided by the alignment lengths.

Hints:

`chomp ($line);` removes the \n character at the end of $line

`$parts[i]` contains the (i+1)s entry of the line.

If $a[11] and $a[3] are variables in an array that contain numbers you can assign a new variable to the ratio using the command

```
$ratio_of_values=$a[11]/$a[3];
```

To print things in a line in the output separated by tabs and a new line symbol at the end you could say for example:

```
print OUT "$line\t$parts[12]\t$ratio\n“;
```

Review Dotlet and Blast (chapter 11).

Caution

NOTE that clustalw and other multiple sequence alignment programs do NOT necessarily find an alignment that is optimal by any given criterion.

Even if an alignment is optimal (like in the Needleman-Wunsch algorithm), it usually is not UNIQUE. It often is a good idea to take different extreme pathways through the alignment matrix, or to use a program like tcoffee that uses many different alignment programs.

clustal

To align sequences clustal performs the following steps:

1) Pairwise distance calculation
2) Clustering analysis of the sequences
3) Iterated alignment of two most similar sequences or groups of sequences.

It is important to realize that the second step is the most important. The relationships found here will create a serious bias in the final alignment. The better your guide tree, the better your final alignment.

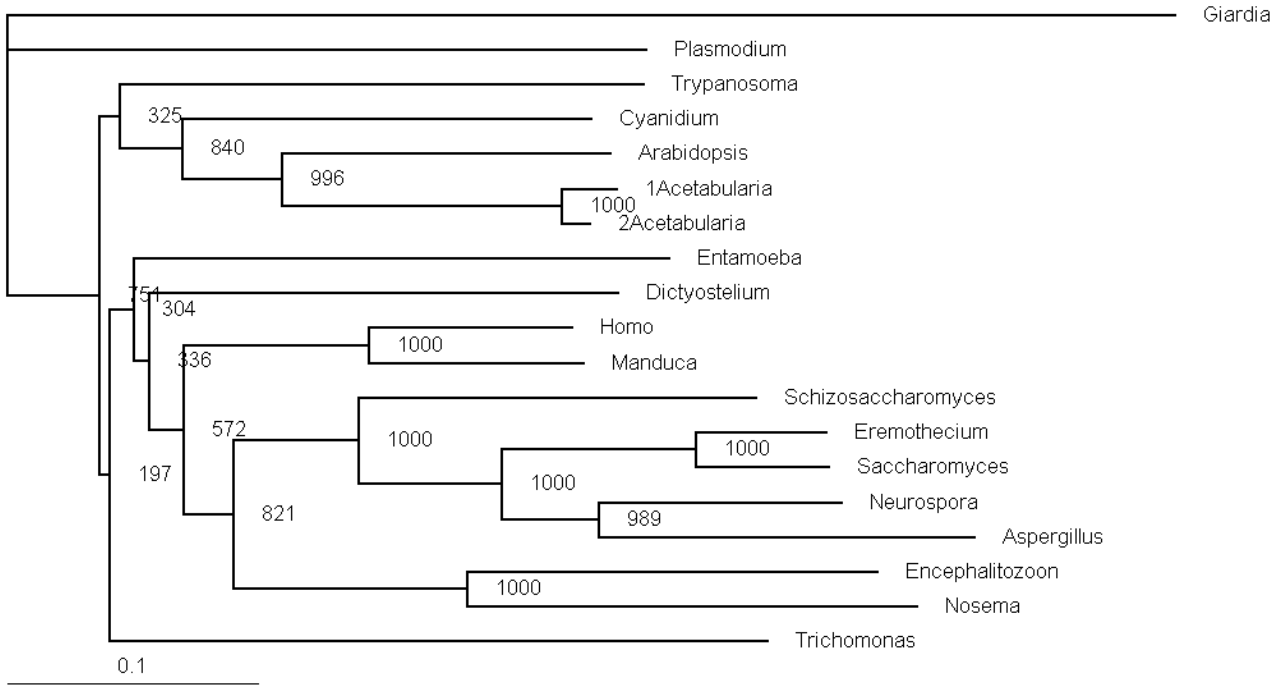
You can load a guide tree into clustal. This tree will then be used instead of the neighbor joining tree calculated by clustalw as a default. (The guide tree needs to be in normal parenthesis notation WITH branch lengths).

Sample input file Sample output file

example on bbcxsv1.biotech.uconn.edu

- calculate multiple sequence alignment
- go through options
- do tree / tree options

  (positions with gaps, correct for multiple subs, support values to nodes, if you want to use treeview)



One way to draw trees on the road is phylodendron

tcoffee

TCOFFEE extracts reliably aligned positions from several multiple or pairwise sequence alignments. It requires more thought and attention from the user than clustalw, but it helps to focus further analyses on those sites that are reliably aligned. A description is here, a web interface is here.

muscle

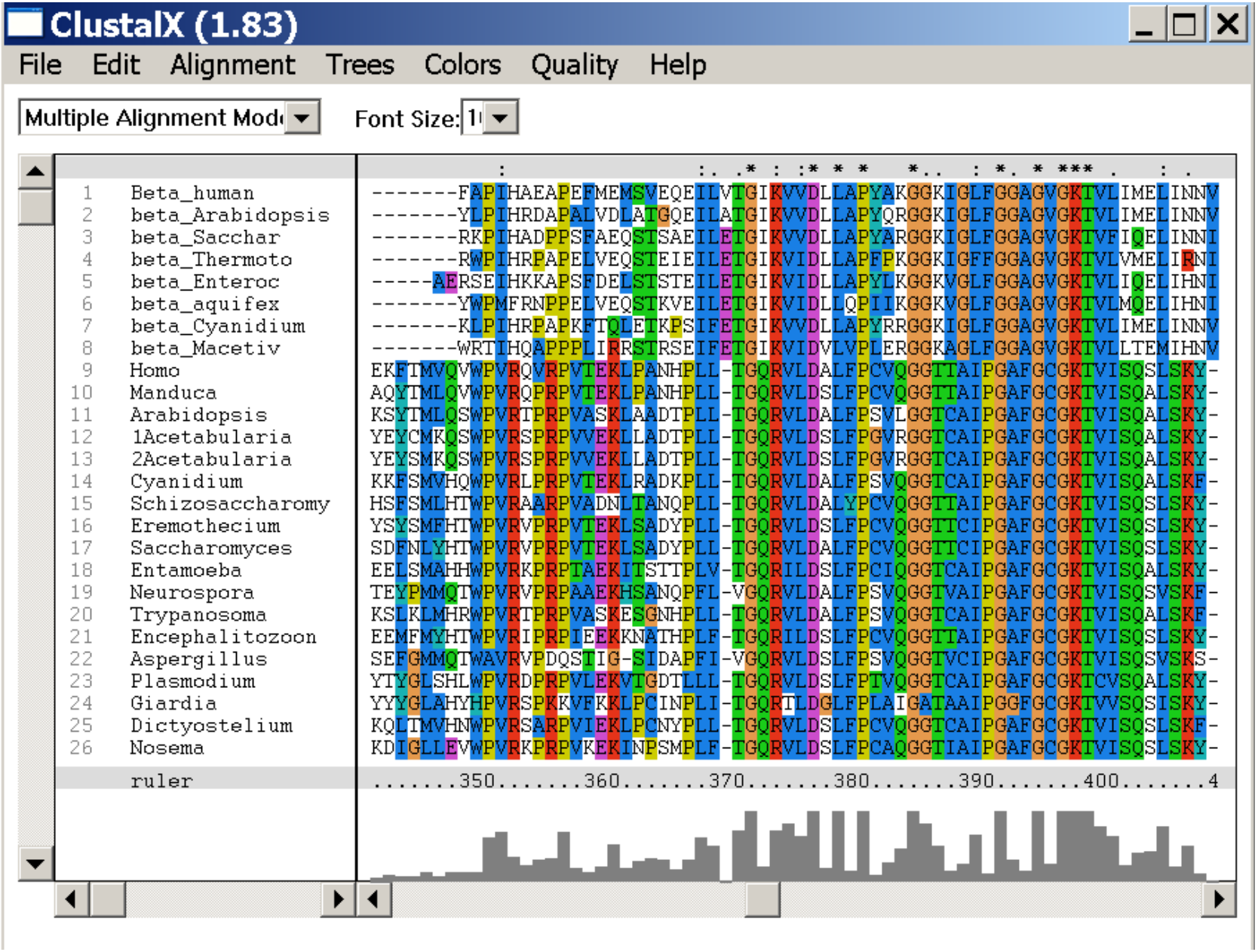
If you have very large datasets muscle is the way to go. It is fast, takes fasta formated sequences as input file, and has a refinement option, that does an excellent job cleaning up around gaps.

The muscle home page is here

Muscle allows also allows profile alignments.

```
muscle -in VatpA.fa -out VatpA.afa
muscle -in VatpA.afa -out VatpA.rafa –refine
muscle -in beta.afa -out beta.rafa -refine
muscle -in beta.afa -out beta.rafa -refine
muscle -profile -in1 beta.rafa -in2
VatpA.rafa -out Abeta.afa
muscle –refine –in Abeta.afa –out Abeta.rafa
```

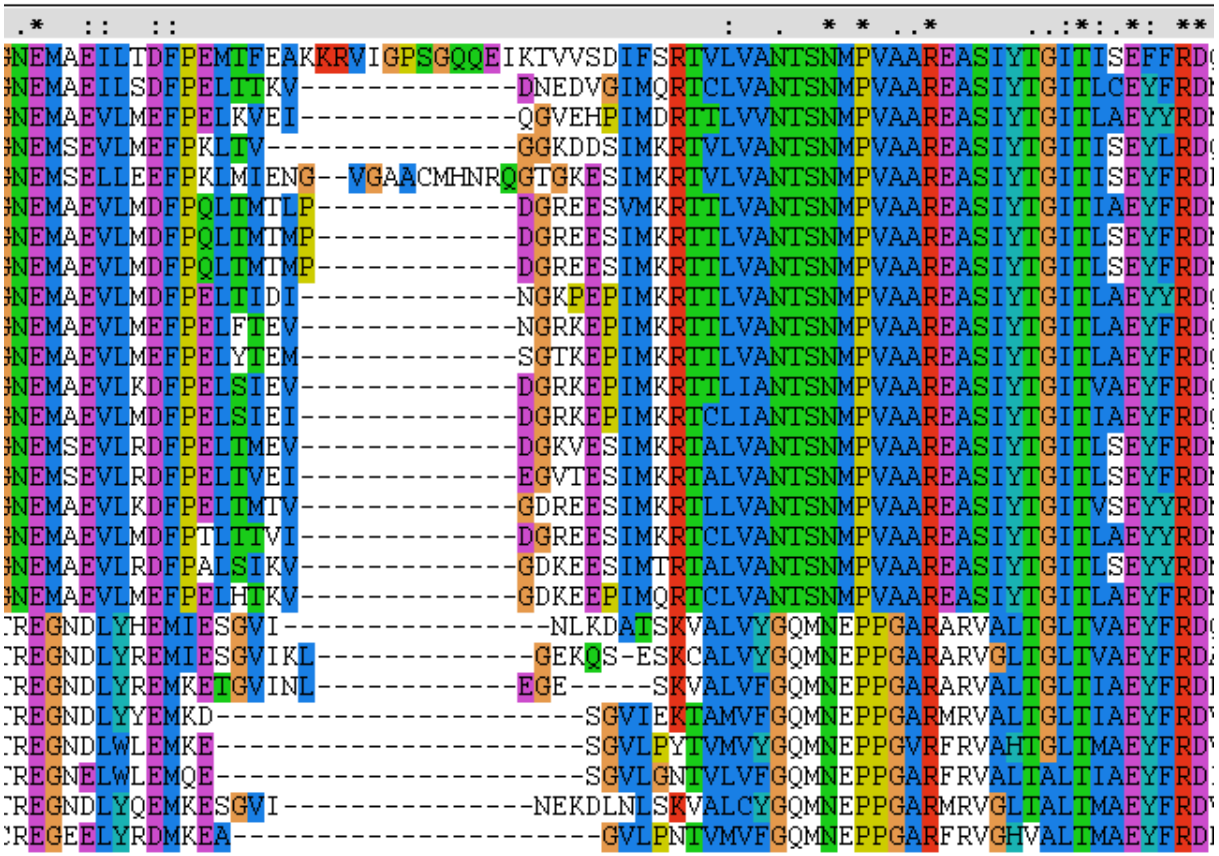
muscle alignment



muscle *vs* clustal

more on alignment programs (statalign, pileup, SAM) here

the same region using tcoffee with default settings



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Sequence editors and viewers

Jalview Homepage, Description
Jalview as Java Web Start Application
(other JAVA applications are here)

Easy to install and run. Test on all.txt (ATPase subunits)
(do 1bmf in spdbv)
(gif of rotation here, movies of the rotation are here and here)
(Load all.txt into Jalview,
colo**u**r options,
mouse use,
PID tree,
Principle component analysis -> sequence space)
Info on sequence space here

seaview – phylo_win

Another useful multiple alignment editor is **seaview**, **the companion sequence editor to phylo_win. It runs on PC and most unix flavors, and is the easiest way to get alignments into phylo_win.**