

Using frames.pl to make a BOXSHADE .rtf file with frame markers

There is some prep work before you start. You will need a FASTA file for each gene containing the sequences for all of that gene's exons. This would look something like:

```
>exon1 (it doesn't matter what is on this line, just that it's here and starts with ">")
ACTGATCATATAGGACATAC...
>exon2 (still doesn't matter)
ATTAGATACCAGATATAGGA...
>etc. for every exon in the gene
TAGACCATGAGATAGACCA...
```

All of that goes in one file, and you'll have a different file for each gene. The sequence must be upper-case, because frames.pl will ignore anything else, and it should *only* include exons - not UTRs. The dots in the example above just mean that there will probably be much more sequence; don't include those. You don't need to supply a full sequence for the gene - frames.pl will put the exons together to generate it for you. Once you have those files, you're ready.

Run frames.pl to generate the multiple alignment, BOXSHADE it, and add the frame lines:

```
perl frames.pl [ -d DIR ] file1 file2 [ file3 ... ]
```

The -d option is optional. If given, the DIR value supplied will be the name of the directory where the files are placed, and will also be used to prefix the file names. If you don't give the option, it will default to "aln". You must then supply the names of the FASTA files for the genes you want to align. You can run as few as 2, or as many as you want above that number.

After you hit enter, frames.pl will ask for a gene name for each file. This is the name that will appear on the multiple alignment, so keep it reasonably short (~3-10 characters). That way the lines won't end up being too long. Finally, you will be asked for an email address for Clustal Omega. Clustal won't email you unless something goes wrong, but it won't start without a valid email address.

Now Clustal will run on your genes, which can take a couple of minutes. If all goes well, you should be informed when Clustal is running, when it generates its many output files, and when it has finished. Next frames.pl will send the Clustal alignment to BOXSHADE to have it shaded, and will apply the frame markers to both alignments. There are now several files in the folder that was created. The prefix "aln" will be different if you gave a value for the -d option. Some of these files are important and some aren't. The ones you may care about are:

```
aln.aln-clustal.clustal
```

This is the multiple alignment that was generated. It does not contain line numbers, which is important because BOXSHADE does not like line numbers.

```
aln.aln-clustal_num.clustal_num
```

This is the multiple alignment *with* line numbers. You won't necessarily need this, but you might want it.

```
aln_frames.txt
```

This is the multiple alignment, once again. This version, however, has the frame markers inserted into it.

aln_boxshade.rtf

This is the output from BOXSHADE, which means it's the same alignment Clustal produced but with the added shading.

aln_boxshade_frames.rtf

This is the BOXSHADE output with frame markers added in.