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
tagaccatgagatagaccaa...
```

All of that goes in one file, and you'll have a different file for each gene. The dots in the sequence just mean that there will probably be much more; 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] exon1 exon2 ...
```

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 (~3-8 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 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_box_frames.txt
```

This file contains the frame lines for all the genes you just entered. If you open it, it will just look like a bunch of weirdly spaced characters, but you will need it for step 3.

`ALN_frames.txt`

This is the multiple alignment, once again. This version, however, has the frame markers inserted into it. If you don't specifically need BOXSHADE, this file may be good enough. If you *do* want BOXSHADE, though, read on.

`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.