

Sequence Verification with A Plasmid Editor (ApE)

1. Obtain version 2.x of ApE from here:

<http://www.biology.utah.edu/jorgensen/wayned/ape/Download/latest%20alphas/>

For Mac, you want the file labeled ApE (Universal).zip

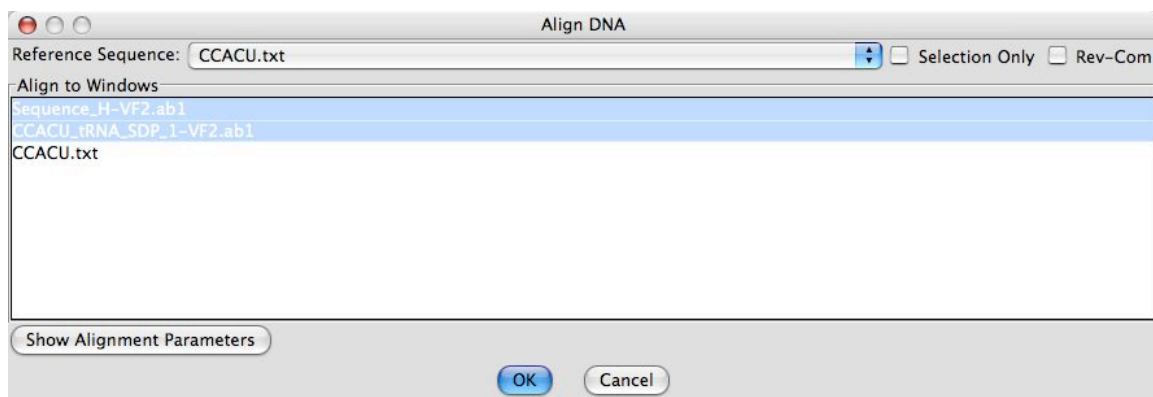
2. Double click on the zipped file to unzip it, then drag the executable file from the disk image.

3. Open ApE.

4. Under File, select Open, and use Command or Shift click to select all your trace (.ab1) files, and a file containing your reference sequence. A separate window should open for each of the files.

5. Go to the reference sequence file. You might want to annotate the sequence with relevant features before proceeding.

6. Under the Tools menu, select Align Sequences... You will see the following window:



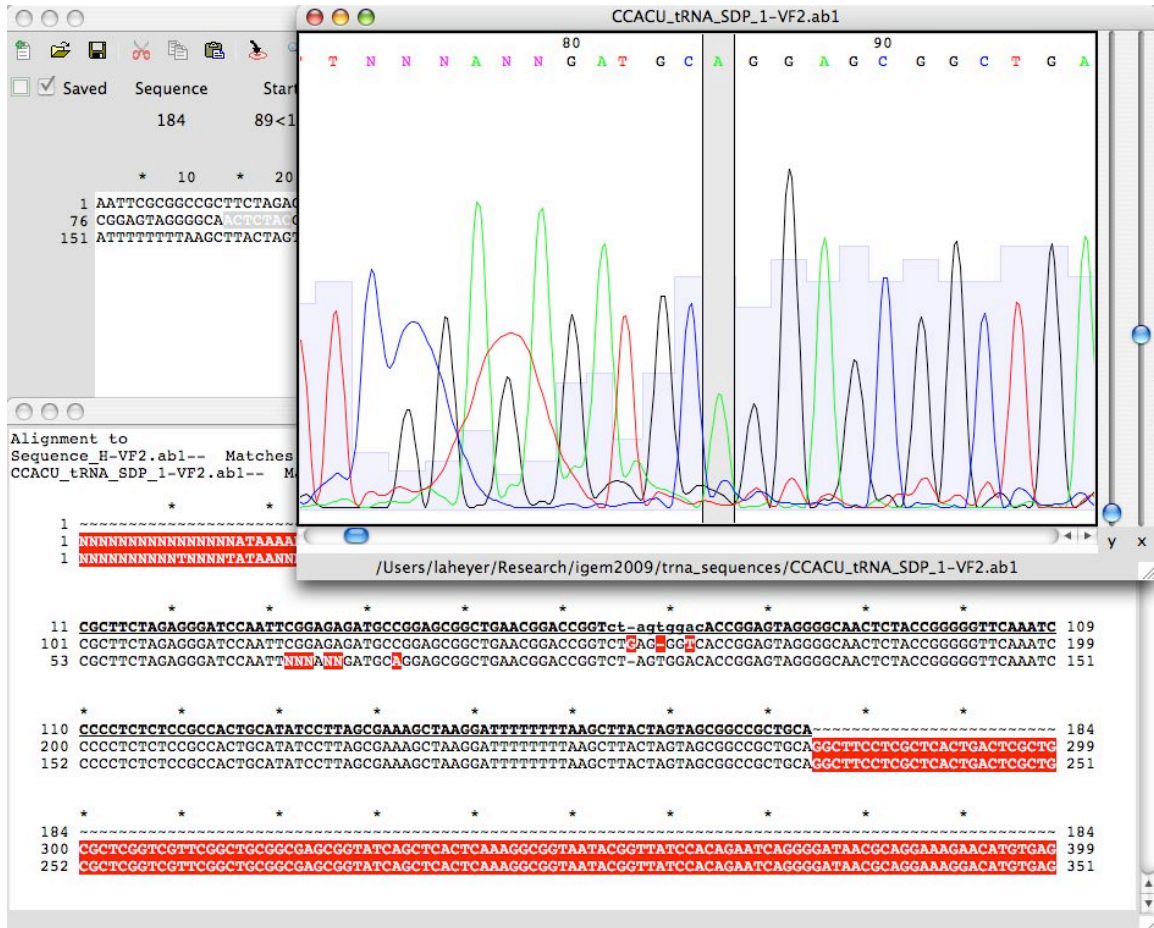
7. Choose the reference sequence and Command click on as many .ab1 files as you want to compare to the reference.

8. Click Rev-Com if you want to align to the reverse complement of the reference sequence.

9. Click the button “Show Alignment Parameters” to reveal further choices. If you have annotated features, you may wish to choose “Copy Highlighting from Sequence” to see those features in the alignment.

9. Click OK to see the alignment. The reference sequence should be on top, and each line below that is a sequence corresponding to the .ab1 file. Mismatches will be marked with red highlighting.

10. Double click on a base in the .ab1 sequence for which you wish to see the trace. The trace will pop up with the selected base highlighted, as shown below.



11. Use the trace heights and bars indicating the quality of the base call to determine how likely it is that you have a point mutation. You can change the x and y scales to space the traces further from each other, or to make them taller or shorter.

12. Double clicking on the corresponding base in the reference sequence line of the alignment window will highlight that base in your original file, helping you judge how important the base is to the function of the molecule.