GENEIOUS PRIME Installation and Use Instructions

Chromebook/Linux users: You will need to download and install a free 14-day trial version of **Geneious Prime** to complete your Lab 7 Vibrio DNA Sequence Analysis activities. Chromebook/Linux users will use Geneious Prime instead of both the 4Peaks and CLC Sequence Viewer apps mentioned in your lab manual. (Geneious Prime can also be used on computers running Mac and Windows operating systems.)

If you have a Chromebook but do not yet have Linux installed, please see this Linux installation guide: https://www.lifewire.com/install-linux-on-chromebook-4125253

Note: *you may need to adjust your computer's Security settings to allow downloaded apps to be opened.*

**A. Geneious Prime installation instructions (free 14-day trial)**

1) Navigate to https://www.geneious.com/academic/free-trial/
2) Enter your information into the form. When you are asked to enter an email address, make sure to use your **berkeley.edu** email address. Click "Get Trial License."
3) Click on the "Download Geneious" button and follow the steps to download the version of Geneious that is appropriate for your computer.
4) Install the program. This process may take a few minutes.
5) Log onto your **berkeley.edu** email and copy the trial license key for Geneious Prime.
6) Launch Geneious Prime. When prompted, click "Activate a License" and paste in the trial license key.
7) If you are prompted to install FlexNet licensing software, go ahead and do so. (Note: if your security settings block FlexNet from being downloaded, you will need to open your System Preferences to allow the FlexNet download.)
8) Your free trial of Geneious Prime should now be activated.

B. Using Geneious Prime for Vibrio DNA Sequence Analysis (Lab Manual pp. 60 - 62)

1) For help navigating in Geneious Prime, you may find it helpful to watch this 3.5-minute orientation video: https://www.youtube.com/watch?v=tl0OwmYJgIc
2) To import sequence files into Geneious Prime:
   a) Open Geneious Prime. If asked whether to allow incoming connections, click "Allow".
   b) If desired, create a new folder for your work in Geneious Prime.
   c) Click and drag your 16S rRNA and *rpoB* sequence files into the document window.

3) ***Reviewing Your Sequences*** (Lab Manual p. 61, Steps 1-8):

   a) Double-click on one of the sequence files to open it in a new window.

   b) Zoom into 100% view to see the nucleotide bases.
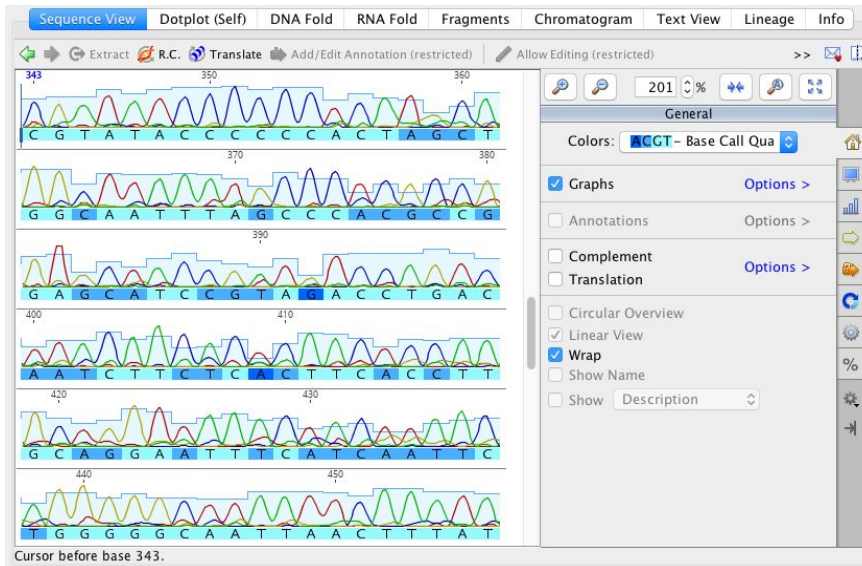
   c) There are light blue columns in the background behind the chromatogram peaks. The height of the blue column shows how confident the program is in the base it has assigned to that position.

   d) Below the peaks you will see the letter of the base that program has assigned to that peak. Each letter will appear in a different shade of blue if "Base Call Quality" coloration is selected in the menu to the right of the main viewing window (General>>Colors>>Base Call Quality). The lighter the blue color, the higher the quality of the base at that point in the sequence. Dark blue represents lower base call quality, while undetermined bases (Ns) show up in red.

   e) Use the quality columns and the base call quality to help you determine where you should delete unreliable nucleotides at the beginning and end of your sequences.

   f) To delete unreliable sequences (Lab Manual p.61 Step 2): select the unreliable nucleotides and then click delete. You may need to click to "Allow editing".

   g) Save changes to your sequence file by going to "*File>>Save as*". Add "trimmed" to the file name to indicate that unreliable nucleotides were trimmed from the sequence.

   h) Repeat for your remaining 16S rRNA and *rpoB* sequences.

4) ***Aligning Your Sequences*** (Lab Manual p. 62)

   a) Geneious Prime does not require you to "flip" or reverse complement your reverse sequence before performing an alignment, so you may skip p. 62 Steps 1-4.

b) To align your forward and reverse sequences, select the 16S rRNA forward and reverse sequence files in the Geneious Prime document window.

c) Click on "Align/Assemble" in the upper menu bar.
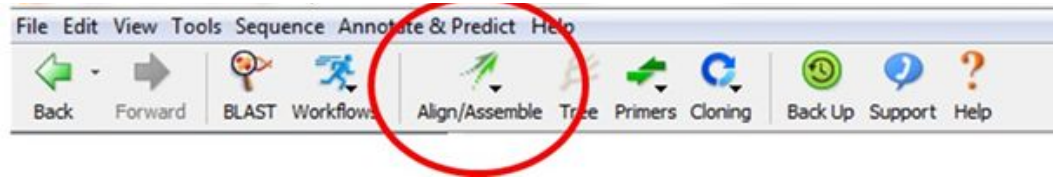
d) Click on "Pairwise Align".

e) In the pop-up dialogue box, keep the default settings (e.g. Geneious Alignment, Global alignment, and 65% similarity). Click the box that says "**Automatically determine direction (slower)**" and then click OK.

f) After you click OK, you will see that the program will create a new document called "Nucleotide alignment." Right-click on this document, select "Edit Name" and give it a name that is more meaningful.

g) Double-click on this new file to open the alignment of your forward and reverse sequences in its own window.

h) We can simplify the alignment view by playing with some formatting options on the right tool bar:
   i) First, **zoom into 100%** view to see the nucleotide bases.
   ii) Under **colors**: select the first rainbow option instead of base call quality.
   iii) **Deselect:** Graphs, Annotations, Highlighting, Translation.
   iv) **Select:** Consensus, Wrap, Show Names.

i) To select the Consensus sequence, left-click on "Consensus". The name "Consensus" and the associated sequence should now be highlighted.

j) Right-click on the highlighted Consensus sequence and click "Copy".

5) ***BLAST Your Consensus 16S rRNA Sequence*** (Lab Manual p.63)
   a) Paste the copied consensus sequence into the BLAST sequence entry window (Lab Manual p. 63 Step 1).
   b) Proceed with the ***BLAST Your Consensus 16S rRNA Sequence*** and ***Building a Phylogenetic Tree Using BLAST*** sections in your lab manual (Lab Manual pp. 63 - 64).
   c) Repeat the above steps to align and BLAST the consensus sequence for the *rpoB* forward and reverse sequences.