16S rRNA tree making guide

1. Make multiple sequence alignment using ClustalW.
http://www.genome.jp/tools/clustalw/

   a. Paste the fasta sequence file (text file) you were provided into the sequence input box or upload it under “General Setting Parameters”.
   b. Under “General Setting Parameters” change the “Output Format” to “Phylip”, select “Slow/Accurate” under “Parawise Alignment”, and select “DNA” next to “Enter your sequences (with labels) below (copy and paste)”. All other settings can remain as default. Click “Execute Multiple Alignment”.
   c. The alignment and associated information should open in the same window. Clicking on “clustalw.phy” on the top of the ClustalW Results page will scroll down to the alignment.
   d. To download the alignment file for the next step, click the “clustalw.phy” link that appears directly above the alignment (not at the top of the page).

2. Make phylogenetic tree
http://www.atgc-montpellier.fr/phyml/

   a. Upload your Phylip formatted alignment next to “Sequences (PHYLIP format)” under “Input Data”.
   b. Enter your email at the bottom of the page “Under Branch Support”.
   c. All default settings remain the same, but you may need to reselect “Data Type” as “DNA” under “Input Data”, “Transition/transversion ratio (DNA models)” as “estimated” under “Substitution Model”, and “Gamma shape parameter” as “estimated” under “Substitution Model” in order for the program to run.
   d. Click “Execute and email results”. When the tree is ready it will be emailed to you. This should only take a few minutes but will depend on the server.
   e. Download and unzip the document attached to the email. It will contain three files.

3. View and modify your tree in the interactive tree of life (iTOL).
http://itol.embl.de/index.shtml

   a. Make an account for iTOL (the Login button is on top right hand corner). Although this may not be necessary, it will allow you to save your work.
   b. To upload your tree from the “username’s trees and data” tab, click “upload a new tree to this project” under the project of your choice.
   c. Upload or paste the contents of the clustalw_phy_phyml_tree.txt file that you downloaded from PhyML. Name your tree and add a description if you want. Then click “upload”. In the next window click “display this tree”.

d. This is your tree! Take some time to change the settings and view your tree in different ways. You must click "Update tree" to view changes you have made.
e. **Changing tree mode:** Under "Basic controls" you can change the "Mode". This will allow you to view your tree as "Unrooted", "Circular", and "Normal". Each mode has advantages and disadvantages depending on what you are focusing on. Change between these modes as needed. Remember to "Update tree" to view changes you have selected.
f. **Changing leave names:** Due to the Phylip format, the names of your 16S rRNA sequences were truncated. To rename these sequences return to the project page in iTOL, by clicking on "username's trees and data" tab. Open the drop down menu for your tree (it's the triangle symbol next to the "Features" column). Select "Re-label leaves". Enter the names manually as they appeared in the original text file of data. You will want to rename your unknown sequence as "unknown #", where # is the unknown sequence number you were assigned. This will allow to you to locate your unknown sequence more easily. Click "Submit changes".
g. **Adding color:** From the project page, open the drop down menu for your tree and select "edit colors". Color the branches for each domain by selecting a color in the "Toolbox" (blue=Bacteria, green=Archaea, red=Eukarya), and then click on the branch of the last common ancestor of the clade you are trying to color (You do not need to name the clades). Click "color clade" (Hint: Eukarya might not be represented as a monophyletic clade. To color this domain, color the whole tree. Then color the other domains). Next color the name of your unknown sequence the color of your choice(a color that stands out) by selecting the color, and then click on the sequence name and selecting "color leave label". Before leaving this page remember to click "save colors and ranges".
h. **Rerooting your tree:** The root of the tree of life was determined by methods other than 16S rRNA phylogenies, so you will need to reroot your tree to display the proper branching order. Display your tree by clicking on its name from the project page. Reroot your 16S rRNA tree at the correct location, by clicking on the appropriate branch and selecting "reroot tree here" in the drop down menu. (Hint: clades will be easiest to see in unrooted mode).
i. **Downloading final tree:** Once you have made the above modifications to your tree put it in "normal" mode and click "export". Use the file format you prefer and click "suggest optimum" next to "Font size". All other options remain default. Then export you tree. Print your tree in color and turn it in with the problem set.