In this practice session we'll use the MEGA package for creating phylogenetic trees.

You can download it through the following link:

https://www.megasoftware.net/

Choose the latest version or an older one, then fill in your details and click on download. We will use version X for this practice.

Let's think about the following scenario:

We have a Canadian patient that got measles after returning from a vacation in Nice, France. We want to identify the source of his infection - was he infected while he was in France? As you know, the measles virus is highly contagious and is spread by coughing and sneezing via close personal contact or direct contact with secretions. In order to better understand the source of the infection for our patient we will construct a phylogenic tree for the Measles virus.

We have sequences of the envelope glycoproteins from the viral surface which is called hemagglutinin (H). They were collected from patients in several locations around the world, including Canadian patients, French patients and South Asian patients. We'll build the phylogenic tree from 5 Canadian patients and 3 patients from Nice in France. Our goal is to know if our Canadian patient was infected by a Canadian or French measles strain. In order to gain confidence in the results of the tree construction and to be able to identify its root, it is advised to add an "outgroup" sequence that should come out as an outlier in the final tree. Therefore, we added the sequence of a Chinese isolate of the virus.

The first stage is to create an MSA. You can either do it within Mega, or import an MSA from a different program such as the clustalO that we previously discussed, as long as they are saved in Mega format with the ".aln" extension.

Here we will align the sequences within Mega: Open the Mega program. Click on align. Choose "edit/build alignment". Choose "create a new alignment" and click OK. If you want to import a MSA choose the option "retrieve a sequence from a file".

At the next window, choose "DNA" for building a DNA sequence alignment.

Now copy and paste your sequences to the opened alignment window (appearing at the end of this exercise).

Do not forget to give informative names for each sequence as these names will be presented at the final phylogenetic tree. So you should edit the description line at the Fasta format sequences by writing the relevant name directly after the bigger than ">" sign. In our example, we added the country name at the beginning of each description line.

The MEGA program offers two commonly used alignment algorithm options: Muscle and ClustlW. Click on the "Alignment" at the main menu and choose "Align by ClustalW (Codons)": This option is used to align (via ClustalW) the coding sequence data in the current selection by first translating all codons to amino acids, performing the alignment, and finally replacing the amino acids with the original codons. Click OK to choose all sequences. At the opened window, use default parameter values and click OK.

Click YES to confirm using that standard genetic code. Now you can scroll on the alignment to see the conserved columns marked with stars (*) and the added gaps.

Next, we need to save the alignment in a Mega format. Click on Data-> Export Alignment ->MEGA format. Give the alignment a name and click Save. At the new window type this name and click OK. Finally, click on “yes” for insuring that these are “protein coding nucleotide sequences”.

Go back to the main program window and click on “Phylogeny”. The program offers a variety of phylogenetic algorithms like Neighbor joining, maximum parsimony, UPGMA and more.

Here we will demonstrate the use of “Neighbor Joining” but as always, we recommend repeating the construction with another algorithm and comparing the results for consistency. Choose the “Neighbor joining” option. Then, choose the alignment file that you saved. In the parameter window change the “test of phylogeny” to “bootstrap method” and write 1000 under “number of bootstrap replication”. Bootstrapping here means that the tree is constructed many times by sampling a partial subset of sequences and the results are tested for internal consistency. You can use the “help” button to get more explanations on each parameter. For example, under the “gaps or missing data treatment” parameter: You may choose to remove all sites containing alignment gaps and missing information before the calculation begins by choosing the “Complete-deletion” option. Alternatively, you may choose to retain all such sites initially, excluding them as necessary in the pairwise distance estimation by choosing “Pairwise-deletion” option, or you may use “Partial Deletion” (Site coverage) as a percentage. We will use the default values recommended. Click on compute.

First, reassuringly we can see that indeed the Chinese gene was clustered separately from all the other ones, suggesting it originated from a different strain. All the Canadian viruses were clustered under the same ancestor meaning they were probably originated from the same strain, whereas all the patients from France were clustered together under another ancestor. We can clearly see that the gene from the Canadian tourist is included in the French group, suggesting that he was infected while he traveling in France.

The numbers next to each node represent the bootstrapping percent number. A high value means that there is strong evidence that the sequences to the right of the node clusters together as it showed up in most of the bootstrap replicates.

We can present just bootstrapping values that are higher than 70 by clicking on View->option-> branches, then clicking on “statistical or frequency” and writing 70 under “hides values lower than”, and clicking OK.

You can also present the tree in different formats. Click on View-> “using tree/branch style” and then choose one of the suggested styles.

The phylogenetic tree can be presented in two ways:

- Phylogram- The branch lengths are proportional to the amount of changes along the branch.

- Cladogram- The branches are of equal length- we can see a common ancestry, but it does not indicate the amount of evolutionary "time" separating taxa.

You can switch between the phylogram and cladogram formats by clicking on view-> topology only.

Let’s color all the French and Canadian patient virus branches in a different color in order to make them distinct from the rest. First click on their common node representing their common ancestor. Click on the hammer sign appearing in the left toolbar. Change the branch line to orange and click OK (note there are a lot of other design options you can use).

We can also add the branch length. The branch lengths usually represent the evolutionary distances between two consecutive nodes. The length of the branch is proportional to the number of changes. The distance between two species is the sum of the length of all branches connecting them.

Click on view, then “show/hide”, finally choose “branch lengths”. In order to remove the branch length, click on “branch length” once more.

And now you know how to create a phylogenetic tree!

>new infected patient

ATGTCACCACAACGAGACCGAATAAATGCCTTCTACAAAGACAACCCCCATCCTAAGGGAAGTAGGATAG

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>FRANCE GQ428193.1 Measles virus genotype D9 strain Mvi/Nice.FRA/20.08/1[D9] hemagglutinin (H) gene, complete cds

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>FRANCE GQ428192.1 Measles virus genotype D9 strain Mvs/Nice.FRA/18.08/2[D9] hemagglutinin (H) gene, complete cds

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>FRANCE GQ428191.1 Measles virus genotype D9 strain Mvs/Nice.FRA/18.08/1[D9] hemagglutinin (H) gene, complete cds

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>CHINA JQ660341.1 Measles virus genotype H1 strain MVi/Zhejiang.CHN/13.10[H1] hemagglutinin gene, complete cds

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>CANADA AF410986.1 Measles virus genotype D7 strain MVs/Alberta.CAN/20.00/1 hemagglutinin gene, complete cds

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>CANADA AF410985.1 Measles virus genotype D8 strain MVi/Montreal.CAN/19.98 hemagglutinin gene, complete cds

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>CANADA AF410975.1 Measles virus genotype D4 strain MVi/Montreal.CAN/12.89 hemagglutinin gene, complete cds

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>CANADA AF410977.1 Measles virus genotype D5 strain MVi/Toronto.CAN/20.96 hemagglutinin gene, complete cds

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>CANADA AF410982.1 Measles virus genotype D6 strain MVi/Vancouver.CAN/13.97 hemagglutinin gene, complete cds

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