- 1. Download your sequences (forward and reverse) from <u>https://ibe.biol.uw.edu.pl/wp-</u> content/uploads/sites/22/2021/09/2021-09-22-4EU_EHB.zip
- 2. Use BioEdit (or any other software that you know how to use) to merge the sequences together into one, more probable sequence.

BioEdit part

- 3. Select the Reverse **fasta** form file name from the left hand side (ex. BR_3.g1) and press **Shift+Ctrl+R** to generate a reverse complement strand. Now the forward and reverse sequences are running in the same direction and have (mostly) the same nucleotides.
- 4. Double click on the file name to the left of the sequence to open a new editing window.
- 5. Highlight and copy the entire sequence (**Ctrl+C**)
- 6. Go to the Forward sequence fasta window. Select "**new sequence**" under the "Sequence" feature in the top tool bar.
 - a. Paste your Reverse sequence in the new window (Ctrl+V)
 - b. Rename this new sequence "R" in the "Name" field.
 - c. Select "DNA" for "Sequence Type" to get the appropriate nucleotide colors.
 - d. Select "Apply and Close." Now both sequences should show up in your Forward window.

Downloading reference sequences

- 7. Once you know what taxon you are working with, download sequences that you think should be on your phylogenetic tree.
 - a. Remember about the outgroup
 - b. Remember to download the correct marker (the same as the one you sequenced).
- 8. Download the sequences from the database (e.g. NCBI) and put them in one multifasta file. Remember to check if the names of the sequences will be understandable and readable when used as leaves on phylogenetic tree. You can use the Mucorales tree as a reference of how the names should look like.
- 9. Add the sequence that you created in the BioEdit part to your multifasta file.
- 10. Use the created multifasta as an input for one-click phylogeny on the phylogeny.fr webpage (<u>http://www.phylogeny.fr/simple_phylogeny.cgi</u>).
- 11. Reroot your tree