Manual for using the eDNAnalyzer program

The software is divided into two parts: “Threshold application” and “Results consolidation”, each requiring a specific input file.

Threshold application

This process will separate the table generated from the taxonomic assignment step, calculate the contigs threshold (default value 0.05%) for each sequencing sample, save a list with the threshold for each sequencing sample, eliminate OTUs below the threshold, and save a new table with the eliminated OTUs.

Input File:

Prepare a table (in .xlsx or .csv format) containing the taxonomic assignment results with the following columns:

- sequencing_sample (identification of sequencing samples)
- area_sampler (indicating the collection area and sampler; ensure that both areas and samplers are unambiguously identified to avoid errors during separation. Use an underscore to separate the area from the sampler: area_sampler.
- point (indicating the collection point)
- n_contigs (number of OTU contigs)
- taxon (taxon determined from taxonomic assignment).

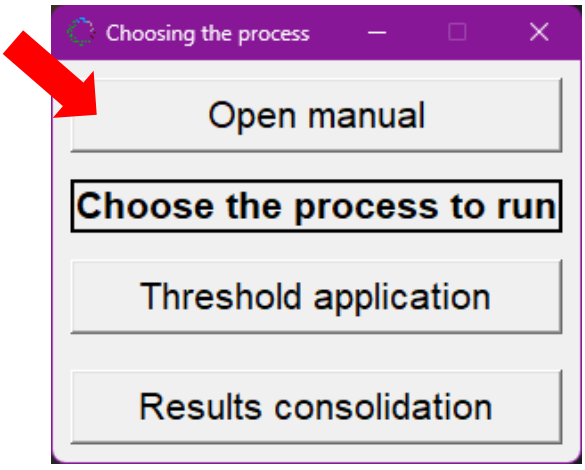
Other columns with different names can also be included, as shown in the following example:

sequencing_sample	tag	area_sampler	point	aliquot	otu	n_contigs	id_%	taxon
C1	TA	A1_MC	1	1	1	89654	100	Alouatta belzebul
C1	TA	A1_MC	1	1	2	542	100	Bos taurus
C1	TA	A1_MC	1	1	3	20	98.45	Bradypus variegatus
C1	TB	A1_MC	1	2	1	75234	100	Homo sapiens
C1	TB	A1_MC	1	2	2	400	99.95	Alouatta belzebul
C1	TB	A1_MC	1	2	3	45	98.98	Coendou prehensilis
C2	TA	A2_MC	2	1	1	47219	100	Homo sapiens
C2	TA	A2_MC	2	1	2	371	100	Bos taurus
C2	TB	A2_MC	2	2	1	65597	100	Sapajus flavius
C2	TB	A2_MC	2	2	2	586	97.56	Coendou sp.
C3	TA	A1_MQ	1	0	1	56076	100	Alouatta belzebul
C3	TA	A1_MQ	1	0	2	692	98.45	Gracilianus agilis
C3	TB	A1_MQ	2	0	1	66490	100	Homo sapiens
C3	TB	A1_MQ	2	0	2	293	98.68	Alouatta belzebul
C3	TB	A1_MQ	2	0	3	198	96.45	Didelphis sp.
C4	TA	A1_AG	1	0	1	79758	99.78	Bos taurus
C4	TA	A1_AG	1	0	2	550	100	Sylvilagus brasiliensis
C4	TA	A1_AG	1	0	3	60	98.45	Bradypus variegatus
C4	TB	A2_AG	1	0	1	71586	100	Homo sapiens
C4	TB	A2_AG	1	0	2	509	100	Alouatta belzebul

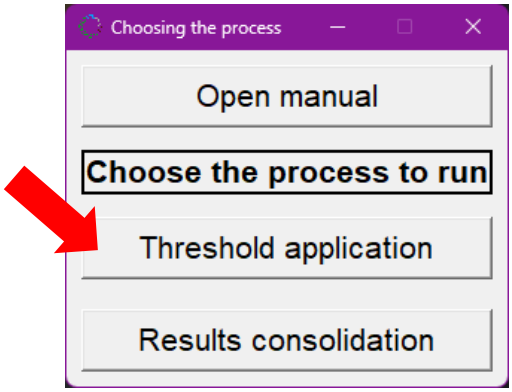
From this point on, open the program and follow these steps:

Step 1: Select the language

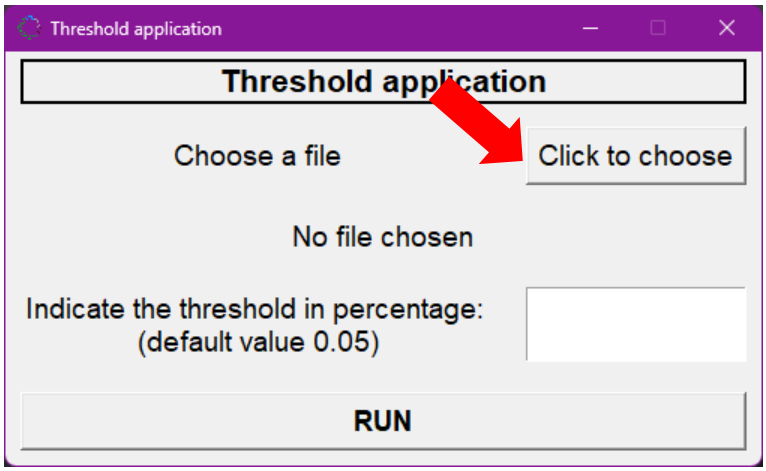
Step 2: You can access the program manual by clicking the "Open Manual" button.



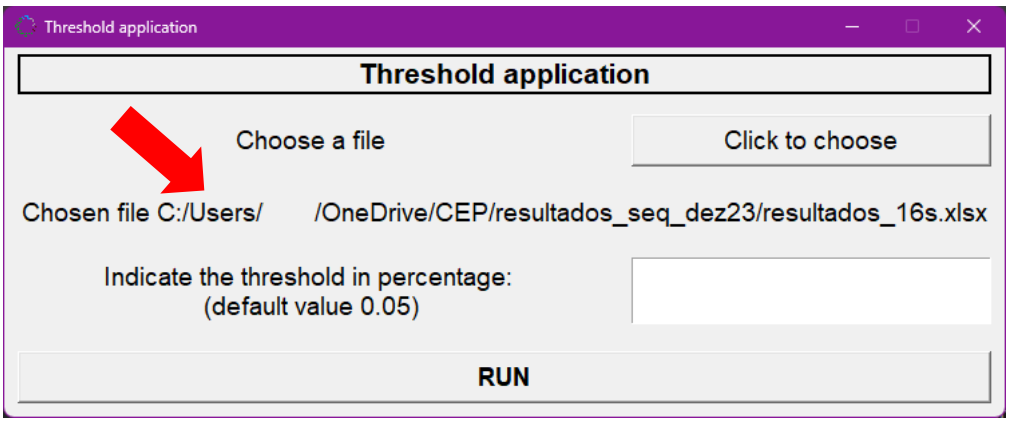
Step 3: Click the "Initial processing and threshold application" button.



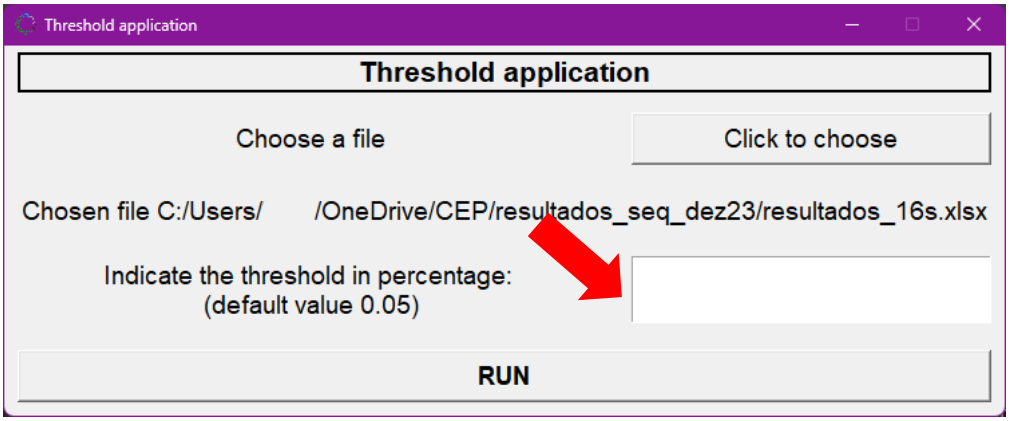
Step 4: Select the table



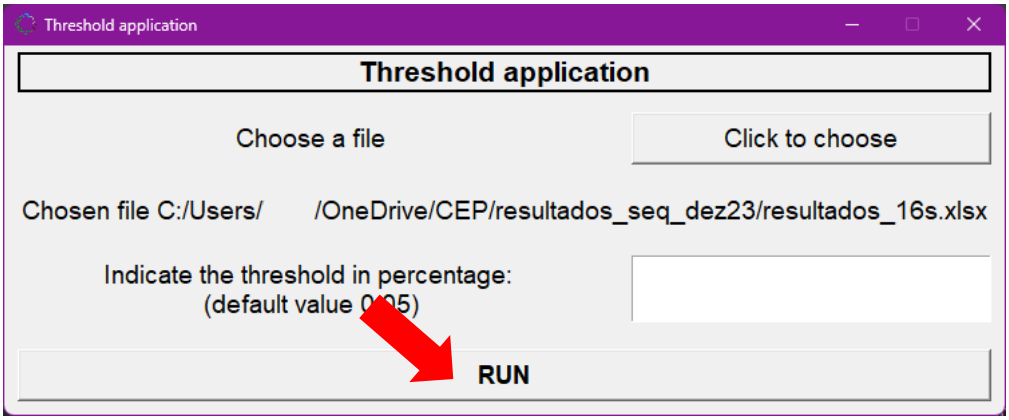
Once you select the file, its path will be displayed. Check to ensure everything is correct.



Step 5: The default value for the threshold is 0.05%, but if you wish to use a different value, please enter it in the text box using a period (.) as the decimal separator.



Step 6: Click on "RUN"



Step 7: Save the table with the processing that was done using the threshold.

Step 4: Save the table with the OTUs that were eliminated.

Step 6: Save the table with the list of thresholds calculated per sequencing sample.

All tables can be saved in .xlsx and .csv formats.

This way, you will have a table like the one used as the input file, but with the removal of OTUs with contig counts below the threshold.

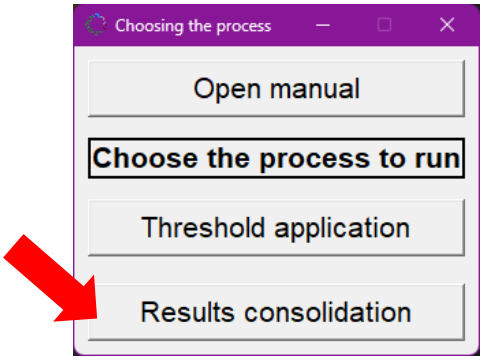
Build table of consolidated results

This process will create tables with the results, lists of species with their number of detections and contigs. The tables can be generated by separating the lists by sampler, by area, or by both.

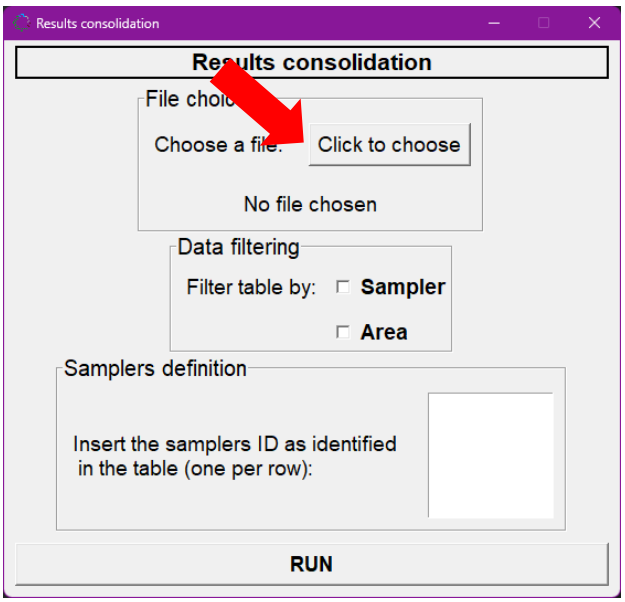
Input file: The input file is the table (in .xlsx or .csv format) from the previous process, slightly modified. A column named **final_otu** should be added, which will contain the final taxonomic assignment after reviewing the results from the previous process.

From here, follow the steps below:

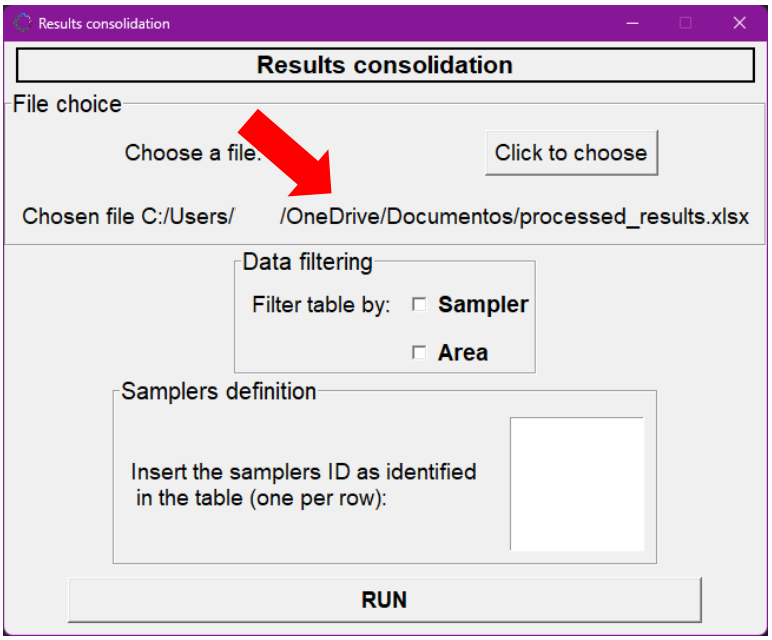
Step 1: Click on the “Build table of consolidated results” button.



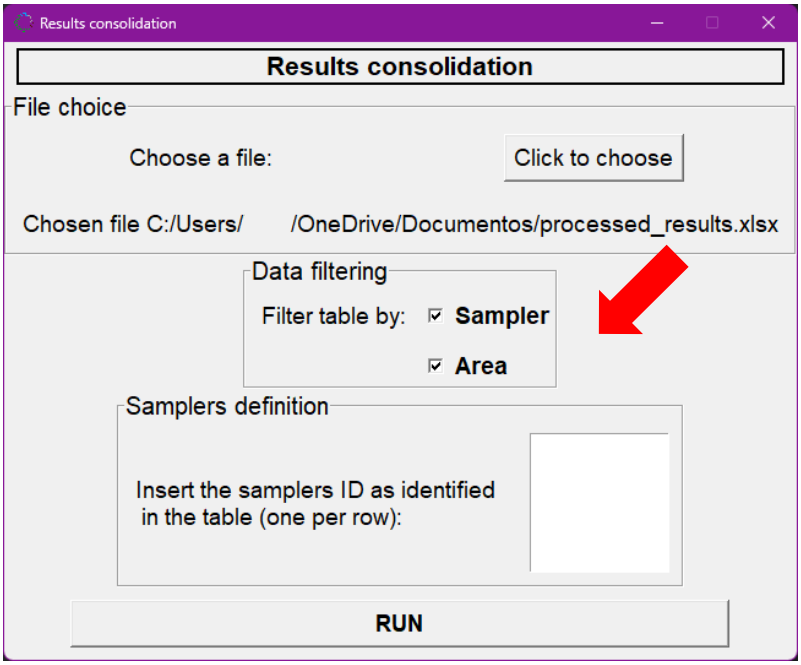
Step 2: Select the table



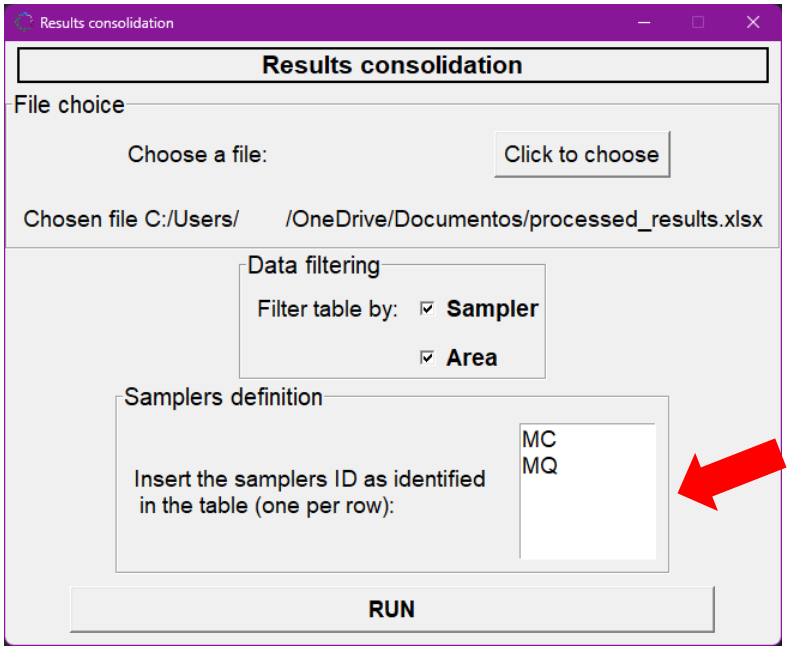
Once you select the file, its path will be displayed. Check to ensure everything is correct.



Step 3: After that, select the criterion by which you want to separate your results: by sampler, by area, or by both.

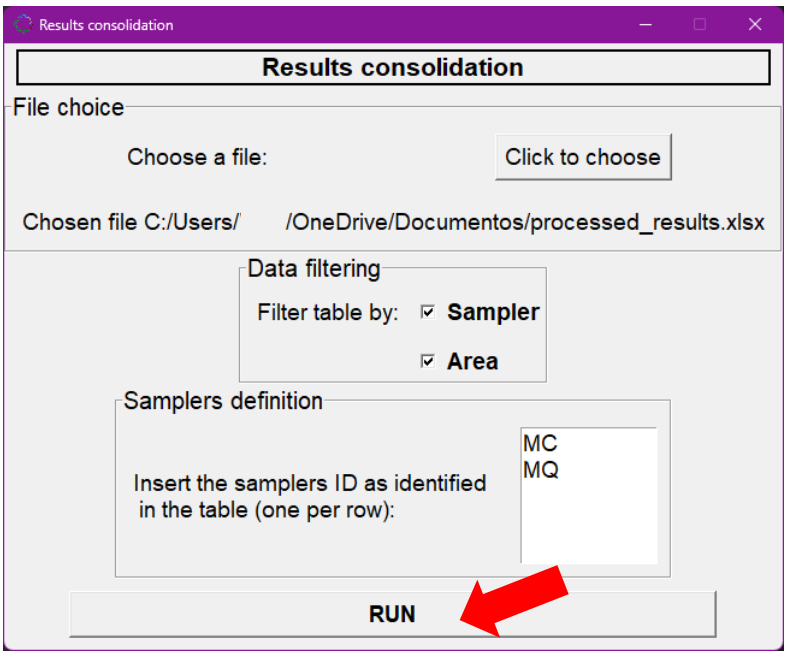


Step 4: If you selected the "Sampler" option, indicate in the next field how you identified your samplers in the table, separating them by pressing Enter, so that they appear one below the other.



If you did not select the "Sampler" option in the previous step, leave this field blank.

Step 5: Click on "RUN"



Step 6: Save the table with a general list of taxa.

Step 7: Save the tables with the final filtered lists according to your choices. To save in .csv format, select the option to save in .zip format so that the tables will be generated in a compressed file.

All tables can be saved in .xlsx and .csv formats.

This way, you will have tables with your final list of taxa, including information about the number of contigs that each taxon presented and how many times it was detected.