eDNAnalyzer Manual

The software is divided into two parts "Initial processing and threshold application" and "Build table of consolidated results", each with a specific input file.

Initial processing and threshold application

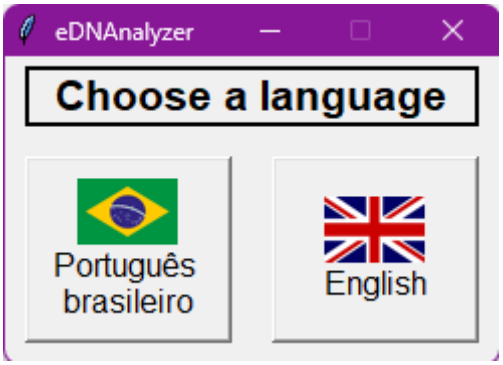
This process will separate the table from the taxonomic assignment step into the sequencing runs, calculate the *threshold* of *reads* (default value 0.05%) for each one, save a list with the *threshold* for each run, eliminate the OTUS that are belowthe *threshold* and save a new table with the eliminated OTUS.

Input file: Set up an Excel table (preferably in .xlsx format) with the results of the taxonomic assignment containing the columns Run (representing the sequencing run), Sample (indicating collection area and sampler, make sure to identify them in an unambiguous way so that there are no errors in the separation, separate area and sampler by an underscore (area_sampler), Point (indicating the collection point), N_reads (number of OTU *reads)* and Species (species defined from the taxonomic assignment). If the samples were separated into aliquots during extraction, include the Aliquot column, with the appropriate identification. There can be other columns in this table with other names without any problem, as shown in the following example:

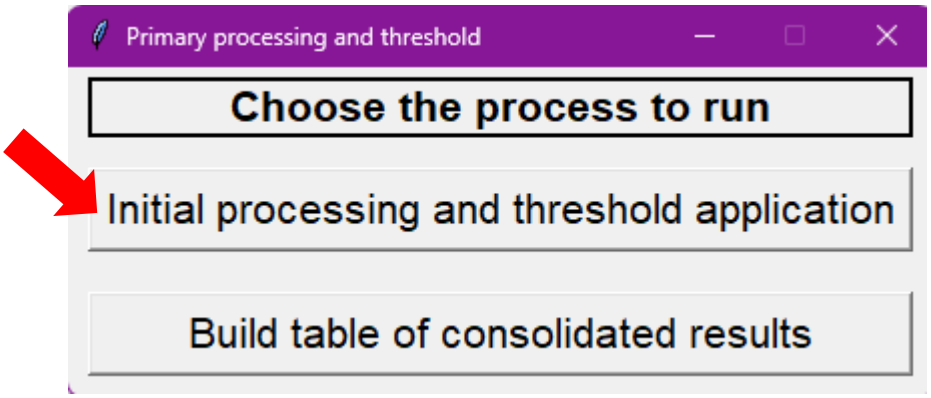
Run	Tag	Sample	Point	Aliquot	Otu	N_reads	id_%	Species
C1	TA	A1_MC	1	1	1	89654	100	Alouatta belzebul
C1	TA	A1_MC	1	1	2	542	100	Bos taurus
C1	TA	A1_MC	1	1	3	20	98.45	Bradypus variegatus
C1	TB	A1_MC	1	2	1	75234	100	Homo sapiens
C1	TB	A1_MC	1	2	2	400	99.95	Alouatta belzebul
C1	TB	A1_MC	1	2	3	45	98.98	Coendou 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 here open the program and follow the steps below:

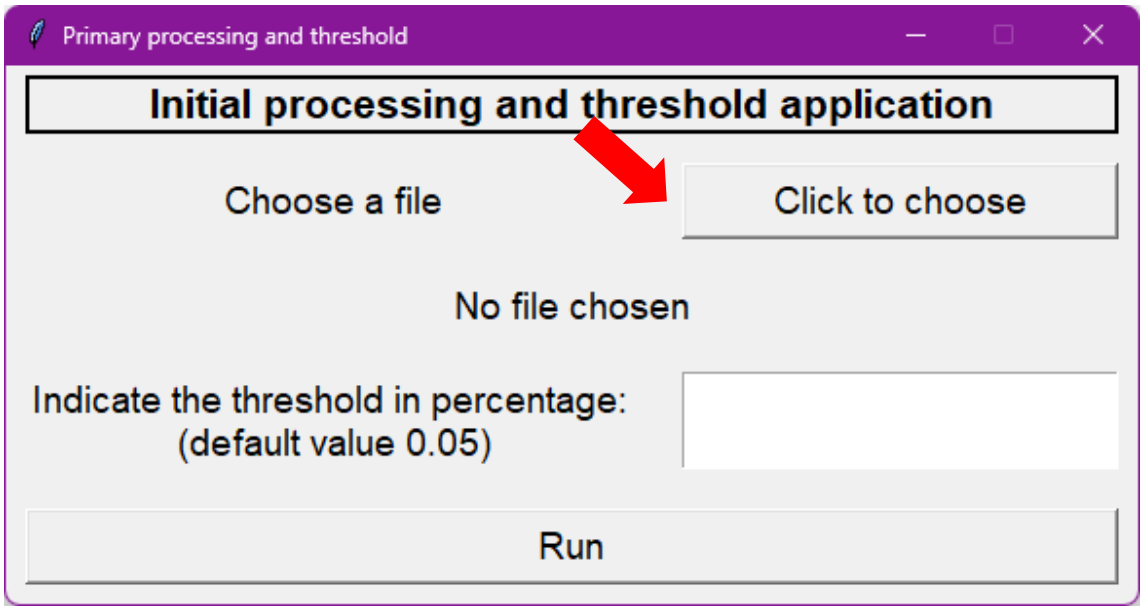
Step 1: Select the language.



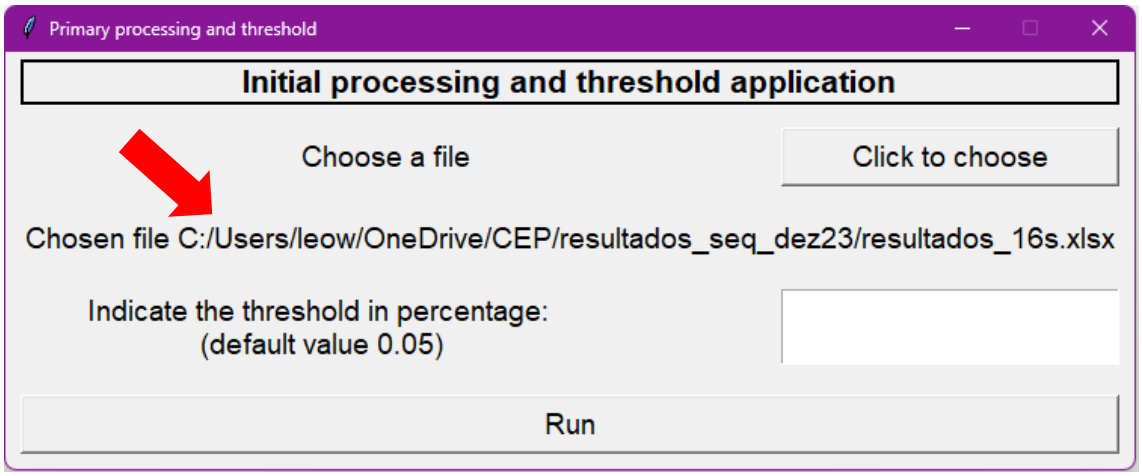
Step 2: Click on the "Processing the initial threshold application" button



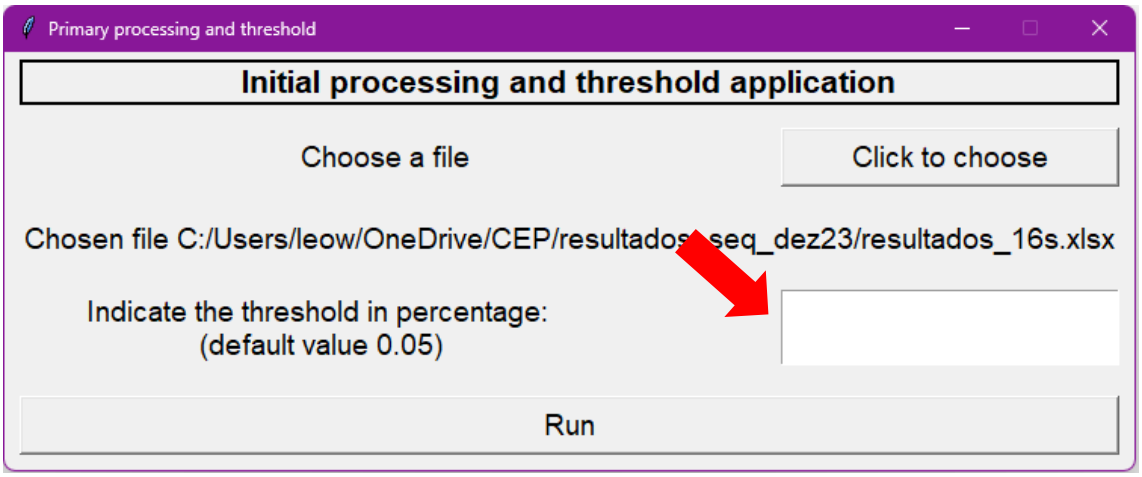
Step 2: Select the table



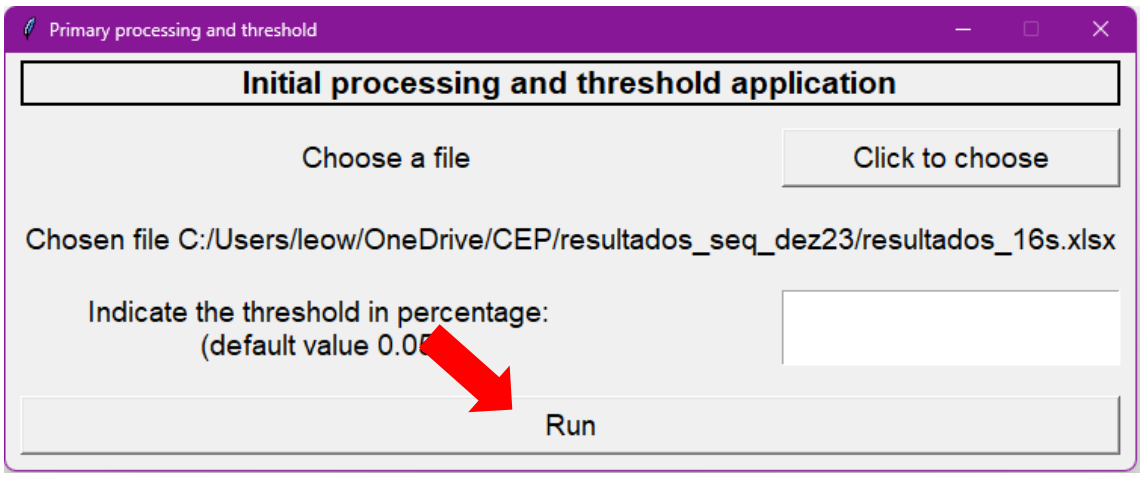
As soon as you select the file, its path will be displayed, check that everything is correct.



Step 3: The default value for the *threshold* is 0.05%, but if you want to use another value.



Step 4: Click on “Run”.



Step 5: Save the Excel table (.xlsx) with the processing that was done using *threshold.*

Step 6: Save the Excel table (.xlsx) with the OTUs that were deleted.

Step 7: Save the Excel table (.xlsx) with the list of *thresholds* calculated per sequencing run.

This way you'll have a table similar to the one you used as an input file, but with the OTUs with a number of *reads* below the *threshold* removed.

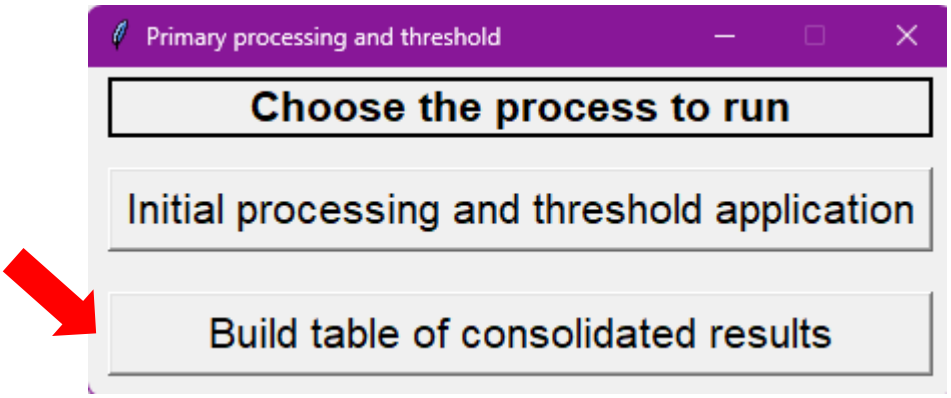
Build table of consolidated results

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

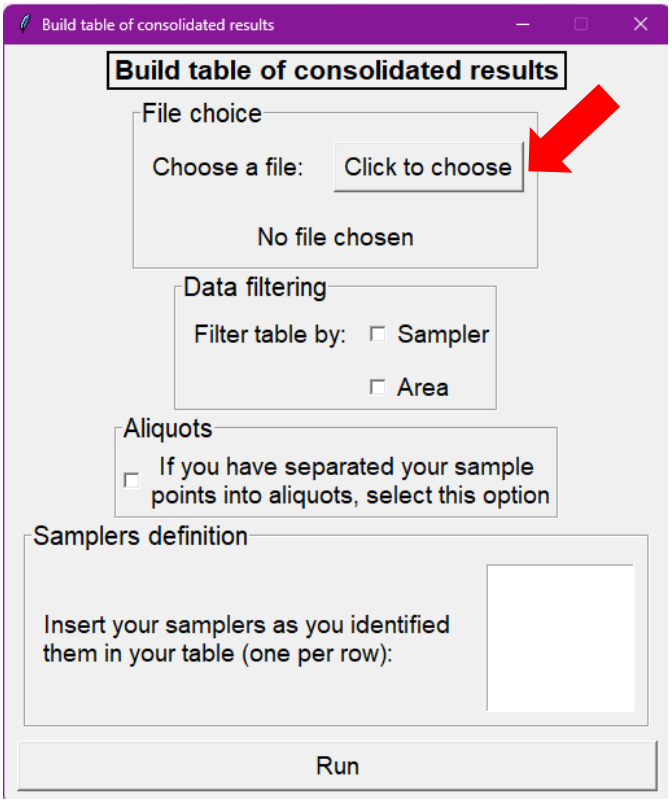
Input file: The input file is the Excel table (preferably in .xlsx format) with the results of the previous process slightly altered, a column named **FinalOTU** should be added, which should contain the final taxonomic assignment after reviewing the results of the previous process.

From here follow the steps below:

Step 1: Click on the "Creating final result tables" button.

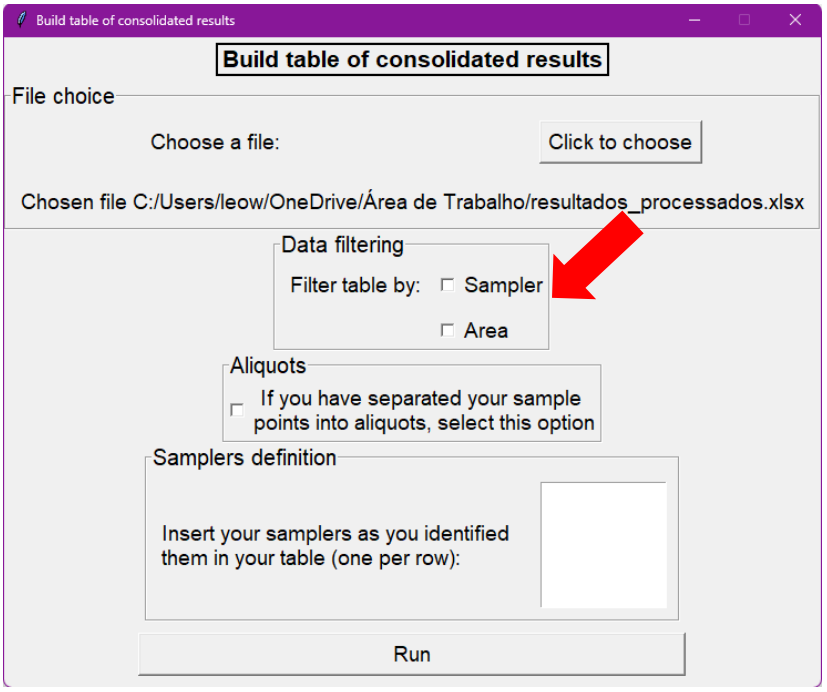


Step 2: Select the table

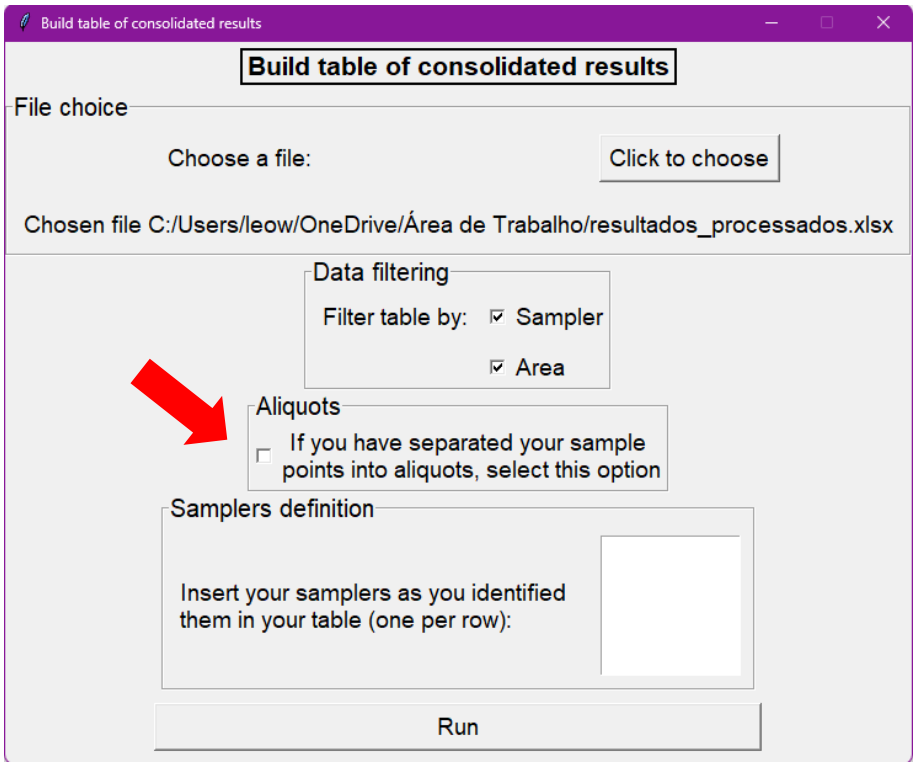


As soon as you select the file, its path will be displayed, check that everything is correct.

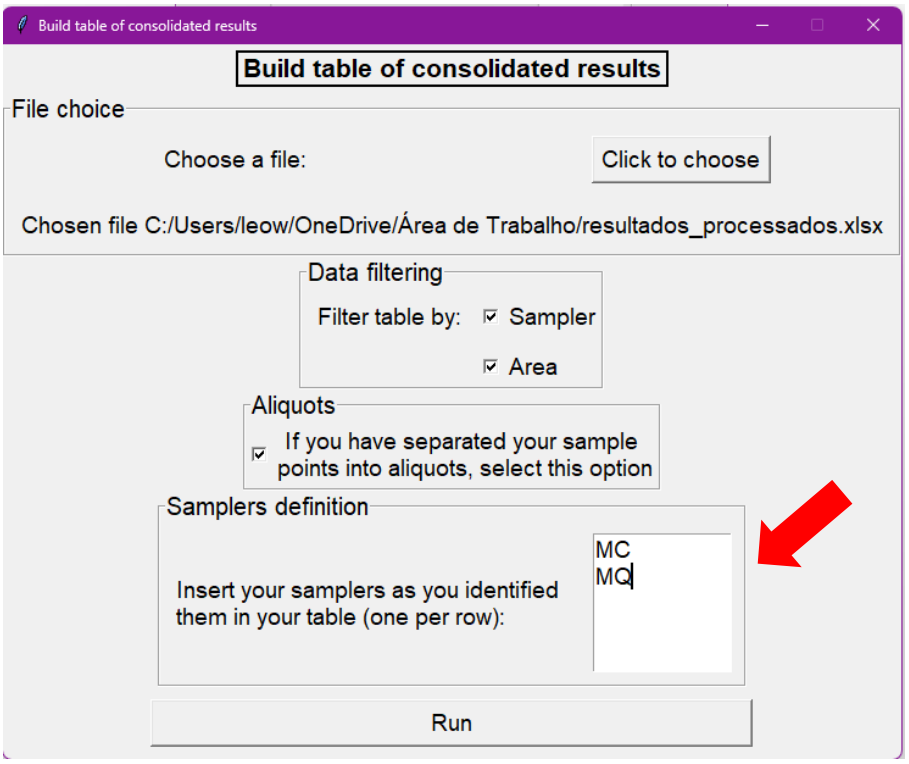
Step 3: After that, select which criteria you want to use to separate your results,by sampler, by area or both.



Step 4: If you used aliquots to extract your samples, select the Aliquots option.

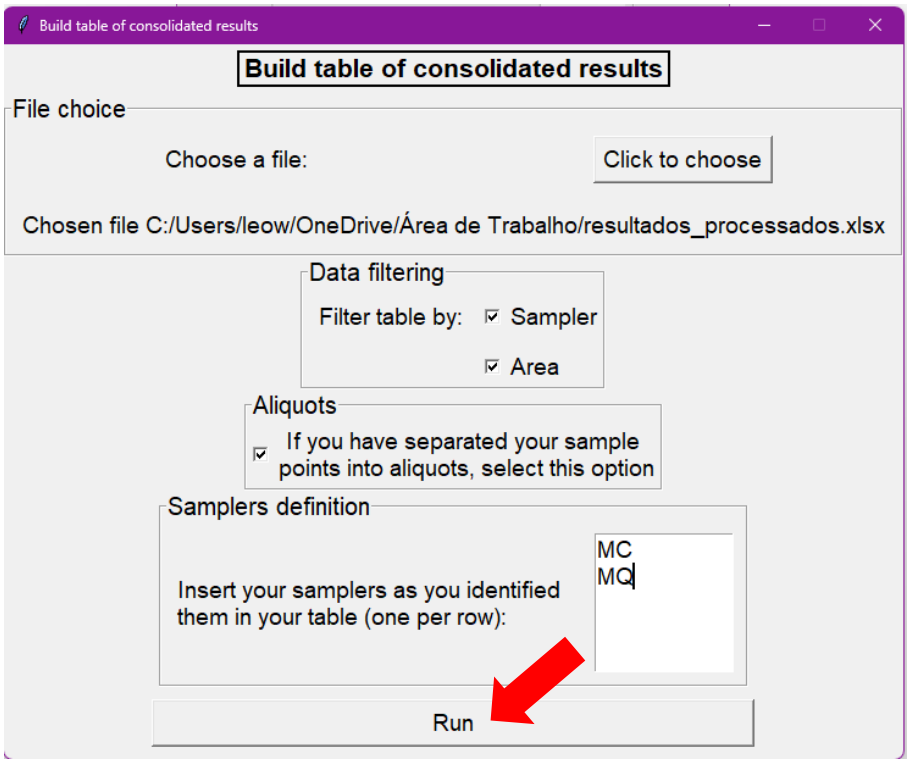


Step 5: If you have selected the "Sampler" option, indicate in the next field how you have identified your samplers in the table, separating them by an Enter, so that they are one below the other.



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

Step 6: Click on "Run"



Step 7: If you chose only one option in **Step 3,** save the table with the final species lists in Excel (.xlsx)

If you chose both options in **Step 3,** save the table with general species lists for each sampler and then the final species lists (a file will be generated per sampler), all the tables generated will be in (.xlsx) format.

This way you'll have tables with your final list of taxa with information on the number of *reads* that taxon had and how many times it was detected.