



Jul 07, 2020

## ♠ Taxonomic classification of IonTorrent-sequenced 16S amplicon sequences

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1 Works for me dx.doi.org/10.17504/protocols.io.bh8pj9vn

DOI

dx.doi.org/10.17504/protocols.io.bh8pj9vn

PROTOCOL CITATION

Laura Espina 2020. Taxonomic classification of IonTorrent-sequenced 16S amplicon sequences.

dx.doi.org/10.17504/protocols.io.bh8pj9vn

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CREATED

Jul 04, 2020

LAST MODIFIED

Jul 07, 2020

PROTOCOL INTEGER ID

38895

DISCLAIMER:

Protocol using QIIME2 pipeline, v2019-4 (doi: 10.1038/s41587-019-0209-9) and the Naïve Bayes classifier implemented in the q2-feature-classifier plugin (doi: 10.1186/s40168-018-0470-z). This classifier was trained on the Greengenes 13\_8 99% OTU database (doi: 10.1038/ismej.2011.139). R v3.6.1 environment was used with Phyloseq v1.29.0 (doi: 10.1371/journal.pone.0061217).

Pre-processing

1



Demultiplex sequences. Quality-filter sequences. Tim barcodes and adapters. Export as fastq files (1 fastq file per sample).



Example of fastq files at this point:

mprotocols.io

07/07/2020

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fcm1\_1\_L001\_R2\_001.fastq fcm2\_2\_L001\_R2\_001.fastq

2 Import fastq files.

Trim primers.

Remove sequences shorter than 100 bp.

Suggested software:



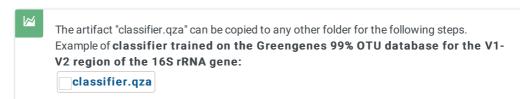
(or python scripts).

3 (Optional) Check the Phred quality score of the sequences. Suggested software:



## On Qiime2

- 4 To train a Naïve Bayes classifier: Download the Greengenes 13\_8 99% OTU database. Upload the files "99\_otus.fasta" and "99\_otu\_taxonomy.txt" onto a folder accesible by the Qiime2 environment. With Qiime2 activated and inside the folder containing those files, type in the command line:
  - $\textbf{4.1} \quad \textbf{>qiime tools import --type 'Feature Data[Sequence]' --input-path 99\_otus.fasta --output-path otus.qza}$
  - 4.2 >qiime tools import --type 'FeatureData[Taxonomy]' --input-format HeaderlessTSVTaxonomyFormat --input-path 99\_otu\_taxonomy.txt --output-path taxonomy.qza
  - 4.3 >qiime feature-classifier extract-reads --i-sequences otus.qza --p-f-primer AGAGTTTGATCMTGGCTCAG --p-r-primer CYNACTGCTGCCTCCCGTAG --o-reads ref-seqs.qza
  - 4.4 >qiime feature-classifier fit-classifier-naive-bayes --i-reference-reads ref-seqs.qza --i-reference-taxonomy taxonomy.qza --o-classifier classifier p.qza



 5 **To perform taxonomy classification**: In a new folder, upload the artifact "classifier.qza" and the file with the metadata corresponding to the fastg files in the correct format. Example of file:

## metadata.tsv

Also create a subfolder "fastq" containing the compressed fastq files in the correct nominal format (eg. "fcm1\_1\_L001\_R1\_001.fastq.qz" and "fcm2\_2\_L001\_R1\_001.fastq.qz") and the classifier artifact. In the command line, type in:

- 5.1 >qiime tools import --type 'SampleData[SequencesWithQuality]' --input-path fastq --input-format CasavaOneEightSingleLanePerSampleDirFmt --output-path demux-single-end.qza
- 5.2 >qiime vsearch dereplicate-sequences --i-sequences demux-single-end.qza --o-dereplicated-table dereplicatedtable.qza --o-dereplicated-sequences rep-seqs.qza
- 5.3 >qiime feature-classifier classify-sklearn --i-classifier classifier.qza --i-reads rep-seqs.qza --o-classification taxonomy.qza
- 5.4 >qiime taxa barplot –i-table dereplicatedtable.qza --i-taxonomy taxonomy.qza --m-metadata-file sample-metadata.tsv --o-visualization taxa-bar-plots.qzv



- 5.5 The components of the biom table (the OTU abundance table and the taxonomy table) can be obtained from the "taxa-bar-plots.qzv" artifact or with the following commands:
  - >qiime tools export --input-path dereplicatedtable.qza --output-path exported-feature-table >cd exported-feature-table
  - >biom convert -i feature-table.biom -o otutable.tsv --to-tsv
  - >qiime tools export --input-path taxonomy.qza --output-path taxonomy



Analysis with R

6 Within R and activating the phyloseq package, different types of analysis can be easily performed.

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- 6.1 The first step is the creation of the phyloseq object using the type of files shown in step 5.5:
  - > otu\_table <- read.csv("otutable.csv",sep=",",row.names=1)
  - > tax\_table <- read.csv("taxonomy.csv",sep=",",row.names=1)
  - > OTU <- otu\_table(otu\_table,taxa\_are\_rows=TRUE)
  - > tax\_matrix<- as.matrix(tax\_table)
  - > TAX <- tax\_table(tax\_matrix)
  - > metadata <- read.csv("metadata.csv",sep=",",row.names=1)
  - > meta <- sample\_data(metadata)
  - > fcm <- phyloseq(OTU, TAX, meta)
- 6.2 Basic operations such as tax\_glom are used to prepare the data for further analyses. For example, with the following commands a table containing the taxonomic abundance for the families present in each sample is created:
  - > fcm.family <- tax\_glom(fcm, "Family", NArm= FALSE)
  - > fcm.family.m <- psmelt(fcm.family)
  - > write.table(x=fcm.family.m, sep = ",", file = "fcm\_family.csv")



At this point a table with the taxonomic abundance of each family is produced. Example of file:

fcm\_family.csv

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