BTW – Bioinformatics Through Windows: an easy-to-install package to analyze marker gene data

Documentation

Installing BTW

BTW package installation instructions can be found at http://www.brmicrobiome.org/tutorialbtw (steps 6 and 7).

Installing the BTW package

Open the installed Ubuntu on Windows.

- In the terminal, type:

```
wget https://raw.githubusercontent.com/vpylro/BTW/master/win_bmp.sh
```

- Then type:

```
sudo bash win_bmp.sh
```

Your UNIX account password will be requested. Type your password and press ENTER. The BTW package will be installed automatically. This may take several minutes, depending on your computer and internet connection speeds.

After finish installing, close the Ubuntu and open it again. The BMP recommended pipeline for 16S rRNA data analyses is available at http://www.brmicrobiome.org/win16s

16S profiling analysis pipeline (Windows)

BMP advisory board recommend the use of this pipeline as a standard for 16S rRNA data analysis.

We are now working in order to improve this workflow besides making it easier for end-users.

If you have any questions or suggestions, please contact

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Please, cite our efforts when using this pipeline: **Data analysis for 16S microbial profiling from different benchtop sequencing platforms. *J Microbiol Methods.* 2014. doi: 10.1016/j.mimet.2014.08.018.**

Please, cite our efforts when using the BTW package: **BTW - Bioinformatics Through the Windows: an easy-to-install package to analyze 16S rRNA data on the Windows Subsystem for Linux (WSL). *(Submitted).***

Also, remember to cite all others softwares applied here.

VSEARCH

QIIME 1.9

FastX Toolkit

fastq-join

FLASH

ClustalW

RDP Classifier

Here, we provide the recommended pipeline for 16S profiling analysis using the Windows Subsystem for Linux (WSL)

What you need: only the BTW package!! Click here

Installing the Windows Subsystem for Linux (WSL): Click here

This example assumes reads in **FASTQ format**.

This page gives a complete pipeline to analyze 16S rRNA gene data. You should edit the commands as needed for your files' addresses (represented here as **$PWD**/).

From Illumina paired-end reads: this pipelines assumes that you have an input folder of paired-up files (by filename, with the default _R1_ and _R2_ containing the forward and reverse reads filenames, respectively):

1 - Take forward and reverse Illumina reads (R1.fastq and R2.fastq files) and join them using the method *fastq-join* **<<<USING QIIME 1.9>>>**

```
multiple_join_paired_ends.py -i input_files -o merged/
```

1.1 - Alternatively, FLASH can be applied to perform the same task (this must be done for each sample, separately)

```
flash -m 20 -M 250 -x 0.25 -p 33  R1.fastq R2.fastq -o merged/
```

2 - Quality filtering, length truncate, and convert to FASTA each joined sample (Attention, the fastqjoin.join files will be inside the $PWD/merged/ folder **<<<USING VSEARCH>>>**

```
vsearch --fastx_filter   $PWD/fastqjoin.join.fastq   --fastq_maxee   1.0 --fastq_trunclen 220 --fastaout samplex.fa
```

Obs: the --fastq_trunclen parameter will depend on the length of your joined reads. You can use **FASTQC** to make this decision.

3 - Change sequence header to make file compatible with further steps **<<<USING BMP PERL SCRIPT>>>**. This script will generate your converted FASTA file. *Sample´s name should not contain any special characters, symbols or spaces. We strongly recommend keeping samples´ names as simple as possible.*

```
bmp_demultiplexed.pl -i samplex.fa -o samplename -b samplename
```

4 - Make a single file containing all your samples

```
cat sample1 sample2 sample3 sample4 ... > reads.fa
```

5 - Dereplication **<<<USING VSEARCH>>>**

```
vsearch --derep_fulllength $PWD/reads.fa --output derep.fa --sizeout
```

6 - Abundance sort and discard singletons **<<<USING VSEARCH>>>**

```
vsearch --sortbysize $PWD/derep.fa --output sorted.fa --minsize 2
```

7 - OTU clustering using UPARSE method **<<<USING VSEARCH>>>**

```
vsearch --cluster_size $PWD/sorted.fa --consout otus1.fa --id 0.97
```

8 - Fasta Formatter **<<<FASTX TOOLKIT SCRIPT>>>**

```
fasta_formatter -i otus1.fa -o formated_otus1.fa
```

9 - Renamer **<<<BMP SCRIPT>>>**

```
bmp-otuName.pl -i formated_otus1.fa -o otus.fa
```

10 - Map reads back to OTU database **<<<VSEARCH>>>**

```
vsearch --usearch_global $PWD/reads.fa --db otus.fa --strand plus --id 0.97 --uc map.txt
```

11 - Assign taxonomy to OTUS using the RDP Classifier **on QIIME** (use the file “otus.fa” as input file)

```
assign_taxonomy.py -i $PWD/otus.fa -m rdp -o taxonomy
```

12 - Align sequences **on QIIME**, using the ClustalW method (use the file “otus.fa” as input file)

```
align_seqs.py -i $PWD/otus.fa -m clustalw -o rep_set_align
```

13 - Filter alignments **on QIIME**

```
filter_alignment.py -i $PWD/otus_aligned.fasta -o filtered_alignment
```

14 - Make the reference tree **on QIIME**

```
make_phylogeny.py –i $PWD/otus_aligned_pfiltered.fasta –o rep_set.tre
```

15 - Convert UC to otu-table.txt **<<< BMP SCRIPT>>>**

```
bmp-map2qiime.py map.uc > otu_table.txt
```

16 - Convert otu_table.txt to otu-table.biom **<<< QIIME SCRIPT>>>**

```
make_otu_table.py -i otu_table.txt -t otus_tax_assignments.txt -o
otu_table.biom
```

17 - Check the OTU Table on QIIME.

```
biom summarize-table –i $PWD/otu_table.biom –o results_biom_table
```

18 - Run diversity analyses **on QIIME** (or any other analysis of your choice). The parameter "-e" is the sequencing depth to use for even sub-sampling and maximum rarefaction depth. You should review the output of the 'biom summarize-table' (step 18) command to decide on this value.

```
core_diversity_analyses.py –i $PWD/otu_table.biom –m $PWD/mapping_file.txt
–t $PWD/rep_set.tre –e xxxx –o $PWD/core_output
```

> **The generated .biom OTU table is also fully compatible with the MicrobiomeAnalyst, a user-friendly web-based platform for microbiome data analyses and visualizations, including taxonomy plots and estimates of α- and β-diversity (http://www.microbiomeanalyst.ca).**

BTW Team

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