

# **Preparatory Instruction**

## **Tutorial: Beginner's guide to microbiome analysis**

### **CSBio2020**

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This instruction is for the “Beginner's guide to microbiome analysis” tutorial, 11th International Conference on Computational Systems-Biology and Bioinformatics (CSBio2020). In order to follow the tutorial smoothly, we provide a preparatory instruction of required hardware, software, datasets, analysis outputs and codes that will be used along the tutorial.

#### **1. Minimum system requirements**

- Processor: Intel Core i5 or AMD equivalent
- Memory (RAM): 8 GB or higher
- Available storage (HDD): 20 GB
- Network accessibility
- Operating systems (OS): Windows 10 or higher, OSX 10.14.x or higher

#### **2. Software requirements and installations**

- Mothur v.1.44.3 (for microbiome analysis)
- SILVA v132 database (for microbiome analysis; taxonomic assignment)
- RStudio v1.3-1093 and required R packages (for diversity analysis and visualization)
- Text editor e.g. Sublime, Visual Studio Code, etc. (for browsing resulting files)

##### **2.1 Mothur [1] and SILVA database v132 [2]**

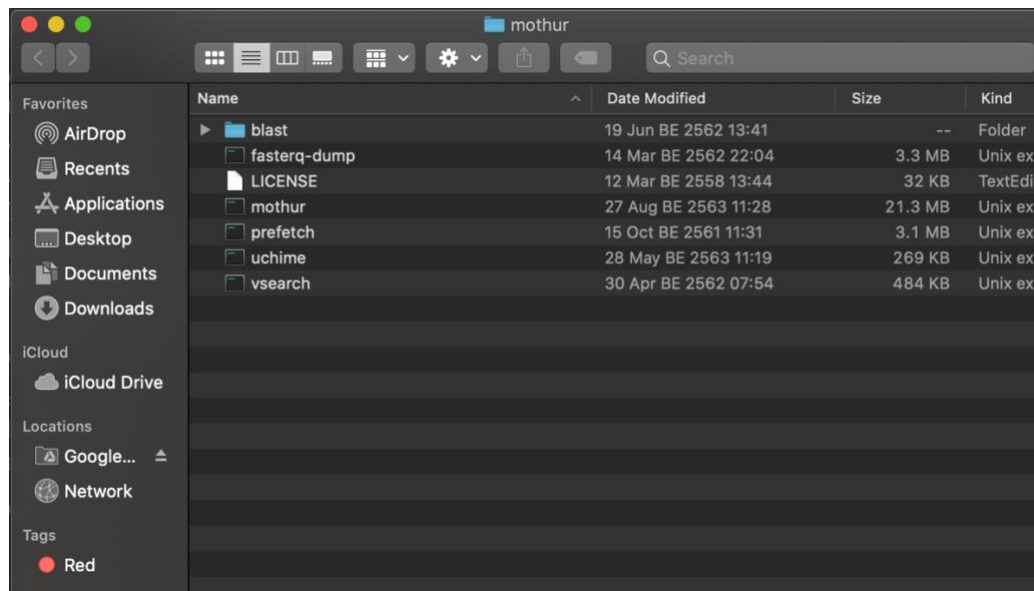
1. Download Mothur.zip file based on your OS (Figure 1). The software is available at <https://github.com/mothur/mothur/releases/tag/v1.44.3>.

▼ Assets 11

|   |         |
|---|---------|
| <a href="#">Mothur.linux.zip</a>            | 19.2 MB |
| <a href="#">Mothur.linux_noReadline.zip</a> | 19.2 MB |
| <a href="#">Mothur.OSX-10.14.zip</a>        | 24.9 MB |
| <a href="#">Mothur.tools_linux.zip</a>      | 12.4 MB |
| <a href="#">Mothur.tools_OSX.zip</a>        | 16.9 MB |
| <a href="#">Mothur.tools_ubuntu.zip</a>     | 12.4 MB |
| <a href="#">Mothur.tools_win.zip</a>        | 9.27 MB |
| <a href="#">Mothur.Ubuntu_18.zip</a>        | 18.9 MB |
| <a href="#">Mothur.win.zip</a>              | 13.8 MB |
| <a href="#">Source code (zip)</a>           |         |
| <a href="#">Source code (tar.gz)</a>        |         |

**Figure 1.** Mothur software installation files. Underlines indicate the installation files for each OS; Linux, OSX, and Windows, respectively.

2. Decompress the downloaded zip file. The ‘mothur’ file in decompressed folder could be used to execute the program (Figure 2).



**Figure 2.** Decompressed mothur folder. The “mothur” program could be executed directly.

3. For taxonomic assignment step, SILVA v132 database will be used. The database for mothur software is available at [https://mothur.s3.us-east-2.amazonaws.com/wiki/silva.nr\\_v132.tgz](https://mothur.s3.us-east-2.amazonaws.com/wiki/silva.nr_v132.tgz).

## 2.2 RStudio and required R packages

1. Before installing RStudio, please make sure that you already installed R. If not please download R from <http://mirrors.psu.ac.th/pub/cran/> and install it first based on your OS (Figure 3).

**The Comprehensive R Archive Network**

**Download and Install R**

Precompiled binary distributions of the base system and contributed packages, **Windows and Mac** users most likely want one of these versions of R:

- [Download R for Linux](#)
- [Download R for \(Mac\) OS X](#)
- [Download R for Windows](#)

R is part of many Linux distributions, you should check with your Linux package management system in addition to the link above.

**Figure 3.** R installation files.

2. Download RStudio installation file and then install program based on your OS (Figure 4). The software available at <https://rstudio.com/products/rstudio/download/>.

| All Installers   |   |           |          |
|--|---|-----------|----------|
| Linux users may need to <a href="#">import RStudio's public code-signing key</a> prior to installation, depending on the operating system's security policy. |   |           |          |
| RStudio requires a 64-bit operating system. If you are on a 32 bit system, you can use an <a href="#">older version of RStudio</a> .                         |   |           |          |
| OS   | Download                                    | Size      | SHA-256  |
| Windows 10/8/7   | <a href="#">RStudio-1.3.1093.exe</a>        | 171.62 MB | 62b9e60a |
| macOS 10.13+   | <a href="#">RStudio-1.3.1093.dmg</a>        | 148.66 MB | bdc4d3a4 |
| Ubuntu 16  | <a href="#">rstudio-1.3.1093-amd64.deb</a>  | 124.33 MB | 72f05048 |
| Ubuntu 18/Debian 10  | <a href="#">rstudio-1.3.1093-amd64.deb</a>  | 126.80 MB | ff222177 |
| Fedora 19/Red Hat 7  | <a href="#">rstudio-1.3.1093-x86_64.rpm</a> | 146.96 MB | ed1f6ef8 |
| Fedora 28/Red Hat 8  | <a href="#">rstudio-1.3.1093-x86_64.rpm</a> | 151.05 MB | 01a978f3 |
| Debian 9   | <a href="#">rstudio-1.3.1093-amd64.deb</a>  | 127.00 MB | a747f9e9 |
| SLES/OpenSUSE 12   | <a href="#">rstudio-1.3.1093-x86_64.rpm</a> | 119.43 MB | 5016cbc7 |
| OpenSUSE 15  | <a href="#">rstudio-1.3.1093-x86_64.rpm</a> | 128.40 MB | cf47e32d |

| Zip/Tarballs        |   |           |          |
|---------------------|---|-----------|----------|
| OS                  | Zip/tar   | Size      | SHA-256  |
| Windows 10/8/7      | <a href="#">RStudio-1.3.1093.zip</a>                  | 253.38 MB | 4abe0f77 |
| Ubuntu 16           | <a href="#">rstudio-1.3.1093-amd64-debian.tar.gz</a>  | 188.97 MB | cc86e368 |
| Ubuntu 18/Debian 10 | <a href="#">rstudio-1.3.1093-amd64-debian.tar.gz</a>  | 189.06 MB | ffc6adaa |
| Fedora 19/Red Hat 7 | <a href="#">rstudio-1.3.1093-x86_64-fedora.tar.gz</a> | 188.66 MB | f37fb229 |
| Debian 9            | <a href="#">rstudio-1.3.1093-amd64-debian.tar.gz</a>  | 189.62 MB | c216d94c |

**Figure 4.** RStudio software installation files. Underlines indicate the installation files for each OS; Windows, and OSX respectively.

3. After installing RStudio, please install all packages needed for data visualization which are ggplot2, dplyr, tidyr, stringr, RColorBrewer, pheatmap, gplots and vegan. For each package, please use a R command inside RStudio to install as:

```
install.packages("PACKAGE_NAME")
```

To activate the package, the command is:

```
library(PACKAGE_NAME)
```

## 2.3 Text editors

A text editor will be used for browsing resulting files during the tutorial. Here we suggest to use Sublime Text or Visual Studio Code software. However, please feel free for any choices. Installation files for the suggested text editors are provided in the following links. Please install and follow the instruction of each software.

- Sublime Text: <https://www.sublimetext.com/3>.
- Visual Studio Code: <https://code.visualstudio.com/download>.

## 3. Datasets

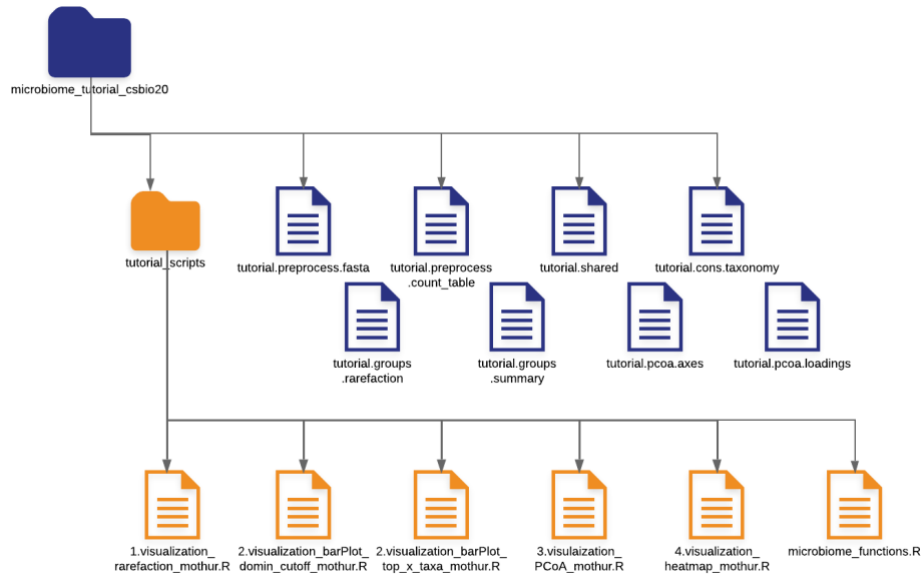
Please download the dataset which will be used in this tutorial at <https://bit.ly/3p85JER>. It is microbiome data of 16S rRNA gene sequences sub-sampled from the study of microbial communities in wastewater treatment systems will be utilized in this tutorial [3]; Data was deposited in European Nucleotide Archive (ENA) under accession number ERP113548. The data is paired-end sequences of V3-V4 hypervariable regions based on Illumina sequencing platform. To provide less time and computational power through the tutorial session, the data were sub-sampled (30%) to reduce the size of the original dataset.

## 4. Analysis outputs

All microbiome analysis outputs are available at [https://github.com/BioM-SBI/microbiome\\_tutorial\\_csbio20](https://github.com/BioM-SBI/microbiome_tutorial_csbio20) (Figure 5). These are outputs that will be produced from the analyses. In case users cannot follow any step during the tutorial session, ones could jump for the output file from each step and continue the next step.

## 5. Codes

All scripts for the microbiome analysis are stored in github and could be downloaded from [https://github.com/BioM-SBI/microbiome\\_tutorial\\_csbio20](https://github.com/BioM-SBI/microbiome_tutorial_csbio20) (Figure 5).



**Figure 5.** Tutorial files architecture. Blue icons indicate analysis files that will be produced/used during the microbiome analysis. Orange icons indicate scripts for the analysis.

## References

1. Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., and Weber, C.F., 2009, "Introducing Mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities", **Appl Environ Microbiol**, Vol. 75, No. 23, pp. 7537-7541.
2. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glockner, F.O., 2013, "The Silva Ribosomal Rna Gene Database Project: Improved Data Processing and Web-Based Tools", **Nucleic Acids Res**, Vol. 41, No. Database issue, pp. D590-596.
3. Puengrang, P., Suraraksa, B., Prommeenate, P., Boonapatcharoen, N., Cheevadhanarak, S., Tanticharoen, M., and Kusonmano, K., 2020, "Diverse Microbial Community Profiles of Propionate-Degrading Cultures Derived from Different Sludge Sources of Anaerobic Wastewater Treatment Plants", **Microorganisms**, Vol. 8, No. 2.