## **MUSiCC User Manual**

MUSiCC is a toolkit for correcting biases in gene abundance measurements derived from shotgun metagenomic sequencing, and is available as an online tool and as a Python module. MUSiCC is developed by the Borenstein group at the University of Washington and is available online at:

http://elbo.gs.washington.edu/software\_musicc.html.

## **MUSiCC>Web**

MUSiCC>Web allows researchers to normalize and correct their metagenomic gene abundance measurements online, and requires only a web browser. The MUSiCC>Web application is built on top of the MUSiCC>Python module.

### Overview of use

To normalize and correct the abundance measurements of genes using MUSiCC>Web, the user simply uploads their gene abundance file to the MUSiCC server by filling out the form shown in Figure 1 a). After checking the integrity of the file, MUSiCC>Web normalizes and corrects the measured abundance. A link to the file containing the corrected abundances is displayed along with statistics of the data and correction procedures, as shown in Figure 1 b).

a)

MUSICC: Metagenomic Universal Single-Copy Correction		
Gene Abundance		
Choose File No file chosen  tab-separated † ?  KEGG Orthology Groups (KO) † ?  Correct inter-sample variation ?		
Correct intra-sample variation Use our generic model 💠		
Correct Abundances		
b)		
Thank you for using MUSiCC! You can download the corrected abundance file here		
Here are some statistics from your data:		
Number of samples	134	
Number of genes	13328	
MUSICC Normalization:		
We corrected inter-sample variation using the set of universal single-copy genens Number of Universal single copy genes found	76/76	
MUSICC Correction:		
We corrected intra-sample variation		
Model used	Generic r	model
Number of genes corrected by model	3912	

Fig. 1: The MUSiCC>Web interface. A) The user is first presented with a form to upload the abundance file and select the analysis options. B) The gene abundance file is corrected and available for download, along with statistics collected during the correction.

## Setting up the analysis

## Selecting the abundance file to correct

*Choose File / Browse*: Select the abundance file on your local drive. In the drop-down box below, choose the appropriate file type.

MUSiCC>Web supports abundance files formatted as plain text with various delimiters. Gene abundance files have one line per gene, with the gene ID appearing as a row header, followed by all the abundance measurements corresponding to different samples separated by a space, comma or tab. Each line must use the same separating character. The first line of the file must contain a column header with sample names.

#### **Analysis Options**

Once you have specified the abundance file, the next step is to choose what types of corrections should be applied to the data:

*Correct inter-sample variation*: Selecting this will perform the normalization step of MUSiCC, correcting inter-sample variation.

**Correct intra-sample variation**: Selecting this will perform the intra-sample correction, with the generic model learned from the Human microbiome project stool samples.

# **MUSiCC>Python**

MUSiCC>Python is a stand-alone Python module that implements the MUSiCC functionality. It is distributed under the GPL and can be readily incorporated into custom analysis tools.

## **Installation Instructions**

#### **Prerequisites for installing:**

In order for MUSiCC to run successfully, the following Python modules should be preinstalled on your system:

- Numpy (http://www.numpy.org/)
- Scipy (http://www.scipy.org/)
- Sklearn (http://scikit-learn.org/stable/)

To install MUSiCC>Python, simply download the package from http://depts.washington.edu/elbogs/MUSiCC/MUSiCC\_Python.zip. This is zip archive containing the following files/directories:

- *MUSiCC.py*: The MUSiCC Python module.
- Data/: A directory containing several data files MUSiCC requires to run properly.
- Example/: A directory containing examples of input and output files.

• *COPYING*: A copy of the GNU General Public License. This is required to be distributed with the MUSiCC>Python package.

### **Interface**

The MUSiCC>Python module handles all calculations internally. MUSiCC>Python offers an interface to the MUSiCC functionality via the command line:

```
usage:
```

```
MUSiCC.py [-h] [-o OUTPUT_FILE] [-if {tab,csv,biom}]
        [-of {tab,csv,biom}] [-n] [-c {use generic,learn model}]
        [-perf] [-v]
        input_file
positional arguments:
                Input abundance file to correct
input file
optional arguments:
 -h, --help
                show this help message and exit
 -o OUTPUT_FILE, --out OUTPUT_FILE
            Output destination for corrected abundance (default:
            MUSiCC.tab)
 -if {tab,csv,biom}, --input_format {tab,csv,biom}
            Option indicating the format of the input file
            (default: tab)
 -of {tab,csv,biom}, --output_format {tab,csv,biom}
            Option indicating the format of the output file
            (default: tab)
                   Apply MUSiCC normalization (default: false)
 -n, --normalize
 -c {use_generic, learn_model}, --correct {use_generic,learn_model}
            Correct abundance per-sample using MUSiCC (default:
```

false)

-perf, --performance Calculate model performance on various gene sets (may add to running time) (default: false)

-v, --verbose Increase verbosity of module (default: false)

## **Example**

In the *Example* directory, the file *Gene\_vs\_Sample\_HMP\_STOOL.tab* contains gene abundance measurements of 5 stool samples downloaded from the Human Microbiome Project (HMP): <a href="ftp://public-ftp.hmpdacc.org/HMMRC/kegg\_kos.pcl.gz">ftp://public-ftp.hmpdacc.org/HMMRC/kegg\_kos.pcl.gz</a>. Using this file as input for MUSiCC results in the normalized and corrected abundance file: *Example/MUSiCC.tab*.

The command used is the following:

Python MUSiCC.py Examples/Gene\_vs\_Sample\_HMP\_STOOL.tab -o Examples/MUSiCC.tab -n -c learn\_model

## **MUSICC>MATLAB**

MUSiCC>MATLAB is a MATLAB function that implements the MUSiCC functionality. It is distributed under the GPL and can be readily incorporated into custom analysis tools.

### **Installation Instructions**

#### **Prerequisites for installing:**

In order for MUSiCC to run successfully, MATLAB needs to be installed on your system.

To install MUSiCC>MATLAB, simply download the package from http://depts.washington.edu/elbogs/MUSiCC/MUSiCC\_MATLAB.zip. This is zip archive containing the following files/directories:

- MUSiCC.m: The MUSiCC MATLAB function.
- Data/: A directory containing several data files MUSiCC requires to run properly.

- Example/: A directory containing examples of input and output files.
- *COPYING*: A copy of the GNU General Public License. This is required to be distributed with the MUSiCC>MATLAB package.

## **Interface**

The MUSiCC>MATLAB module handles all calculations internally. MUSiCC>MATLAB offers an interface to the MUSiCC functionality via the MATLAB environment:

#### usage:

>> CorrectedAbundance = MUSiCC(abundance file, varargin)

#### required input:

abundance file

Input abundance file to correct

#### optional input:

output\_file <str>: name for the output file (default: MUSiCC.tab)

normalize <'true'/'false'>: Determines if MUSiCC normalization is performed (default 'true')

correct\_method <'use\_generic'/'learn\_model'>: Determines if MUSiCC correction is performed (default 'none')

show\_scores <'true'/'false'>: Determines if MUSiCC reports scores for variuos gene sets (default 'true')

verbose <'true'/'false'>: Determines if MUSiCC increases verbosity (default 'true')

#### **Output:**

CorrectedAbundance: the corrected gene abundance matrix

## **Example**

In the *Example* directory, the file *Gene\_vs\_Sample\_HMP\_STOOL.tab* contains gene abundance measurements of 5 stool samples downloaded from the Human Microbiome Project (HMP): <a href="ftp://public-ftp.hmpdacc.org/HMMRC/kegg">ftp://public-ftp.hmpdacc.org/HMMRC/kegg</a> kos.pcl.gz. Using this file

as input for MUSiCC results in the normalized and corrected abundance file: Example/MUSiCC.tab.

The command used is the following:

>> MUSiCC('Examples/Gene\_vs\_Sample\_HMP\_STOOL.tab', 'output\_file', 'Examples/MUSiCC.tab', 'correct\_method', 'learn\_model')