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MicroMPN: Software for Automating Most Probable Number Estimates from Laboratory Microplates

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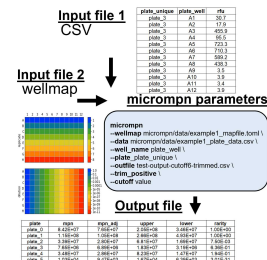
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We use this protocol and it's working.

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Abstract

We developed a command-line Python package named "MicroMPN" to enable the automation of most probable number (MPN) value estimations from microplates of different formats and sample designs. In this protocol, we guide users on how to run MicroMPN from a Jupyter Notebook using simulated fluorescence data values. To run MicroMPN, the users must input two data files. Input file 1: the user provides a CSV file with the plate name, the well locations of each sample in a microplate, and the spectrofluorometric data. Input file 2: the user specifies the layout of a microplate with a Wellmap TOML-based format file. The second file contains the number of replicates, the dilution series, and the number of samples per plate. The Python package "wellmap" parses the information of a microplate from the TOML formatted file for downstream calculation of an MPN value. Additionally, users must specify a cutoff/threshold value, which is used to determine which wells are positive or negative for microbial growth. Output file: MicroMPN will calculate an MPN value, a bias-corrected MPN value, 95% confidence intervals, and a rarity index.

Guidelines

- The most probable number (MPN) assay is a culture-dependent method of quantification most commonly used to enumerate microbes from environmental samples ¹. In the MPN assay, the density of viable microbes is determined by serially diluting to extinction in a culture medium. An MPN count (MPN/mL or MPN/g) is therefore based on the presence or absence of a measurable signal, such as media turbidity ², color change ^{3,4}, and luminescence ⁵⁻¹⁰. MPN calculations require two fixed quantities: sample volume and number of replicates at each dilution step. Calculations of an MPN value are based on probability theory and not on an absolute count ¹¹. A Poisson distribution is used to evaluate the likelihood of microbes being transferred (or not) in a dilution step, based on the inoculum concentration. Given the probability of a transfer occurring, a binomial distribution is then used to evaluate the likelihood of observing a specific number of positive replicates in a dilution step ¹¹. The initial concentration that maximizes the combined likelihood of all dilution steps is the most probable number of microbes in the sample.
- A portion of the code used in MicroMPN was derived from the "MPN" R package created by Ferguson and Ihrie (2019) ¹⁶.
- As an alternative to our command-line tool, there are web-based MPN calculators where users manually input their values. One is the Environmental Protection Agency MPN calculator (<https://mostprobablenumbercalculator.epa.gov/mpnForm>). Another is MPNCalc v1.2.0 (<https://mpncalc.galaxytrkr.org/>) created by M. Ferguson and J. Ihrie at the Food and Drug Administration.
- Additionally, users can use the R package "MPN" (written by the same authors that created MPNCalc), whose code we transpiled to Python to run MicroMPN from the terminal.

Materials

A computer, internet access, and software MicroMPN.

Before start

In this protocol we cover how to run MicroMPN from a Jupyter Notebook.

However, users that choose to run the package from a terminal can access MicroMPN through:

Github:

```
git clone git@github.com:USDA-ARS-GBRU/micrompn.git
cd micrompn
pip install .
```

PyPI:

```
pip install micrompn
```

docker:

```
docker pull ghcr.io/usda-ars-gbru/micrompn:main
```

Example of Input file 1: namefile.csv

- 1 To run MicroMPN, users need an input CSV file with RFU data.

The column headers must include the following identifiers:

plate_unique

plate_well

rfu

**Additional column names in the input file will be ignored by MicroMPN.*

As an example of a CSV file with relative fluorescence units (RFU) data, we provide users with  input_protocols_io.csv. The input file provided contains RFU data in a 12 (dilution series) by 8 (replicates) microplate layout. In this microplate, column 1 has 8 replicates of resuspended soil sample. Columns 2 - 12, contain 10-fold serially diluted samples. Values correspond to spectrofluorometric measurements collected 48 h after incubating the plate.

C	D	E	F
plate_unique	plate_id	plate_well	rfu
plate_3	RFP_1_plate_1	A1	30.731
plate_3	RFP_1_plate_1	A2	17.946
plate_3	RFP_1_plate_1	A3	455.882
plate_3	RFP_1_plate_1	A4	95.484
plate_3	RFP_1_plate_1	A5	723.326
plate_3	RFP_1_plate_1	A6	710.278
plate_3	RFP_1_plate_1	A7	589.153
plate_3	RFP_1_plate_1	A8	438.318
plate_3	RFP_1_plate_1	A9	3.51
plate_3	RFP_1_plate_1	A10	3.889
plate_3	RFP_1_plate_1	A11	3.435
plate_3	RFP_1_plate_1	A12	3.949
plate_3	RFP_1_plate_1	B1	31.577
plate_3	RFP_1_plate_1	B2	15.791
plate_3	RFP_1_plate_1	B3	344.181
plate_3	RFP_1_plate_1	B4	645.707
plate_3	RFP_1_plate_1	B5	659.22
plate_3	RFP_1_plate_1	B6	609.989
plate_3	RFP_1_plate_1	B7	550.889
plate_3	RFP_1_plate_1	B8	3.987

Example of Input file 2: namefile.toml (namefile.txt)

- 2 To run MicroMPN, users need a second input file which specifies the layout of samples across a microplate. A Wellmap file can be created using a text editor (e.g., notepad). The format of the file is TOML-based.

```
[col]
1.dilution = 1e00
2.dilution = 1e-01
3.dilution = 1e-02
4.dilution = 1e-03
5.dilution = 1e-04
6.dilution = 1e-05
7.dilution = 1e-06
8.dilution = 1e-07
9.dilution = 1e-08
10.dilution = 1e-09
11.dilution = 1e-10
12.dilution = 1e-11
```

```
[row]
A.replicate = 1
B.replicate = 2
C.replicate = 3
D.replicate = 4
E.replicate = 5
F.replicate = 6
G.replicate = 7
H.replicate = 8
```

```
[block.12×8.'A1']
sample = 1
```

In this .toml file  microplate.toml , each row (A – H) was assigned a replicate value (1 – 8), while each column (1 – 12) represented a particular dilution factor (1e00 – 1e-11). For this reason, each plate was divided into a single 12×8 sample block, although multiple sample blocks on a plate are possible. A .toml file can be opened with a text editor (e.g., notepad).

Example of how to run MicroMPN from a Jupyter Notebook

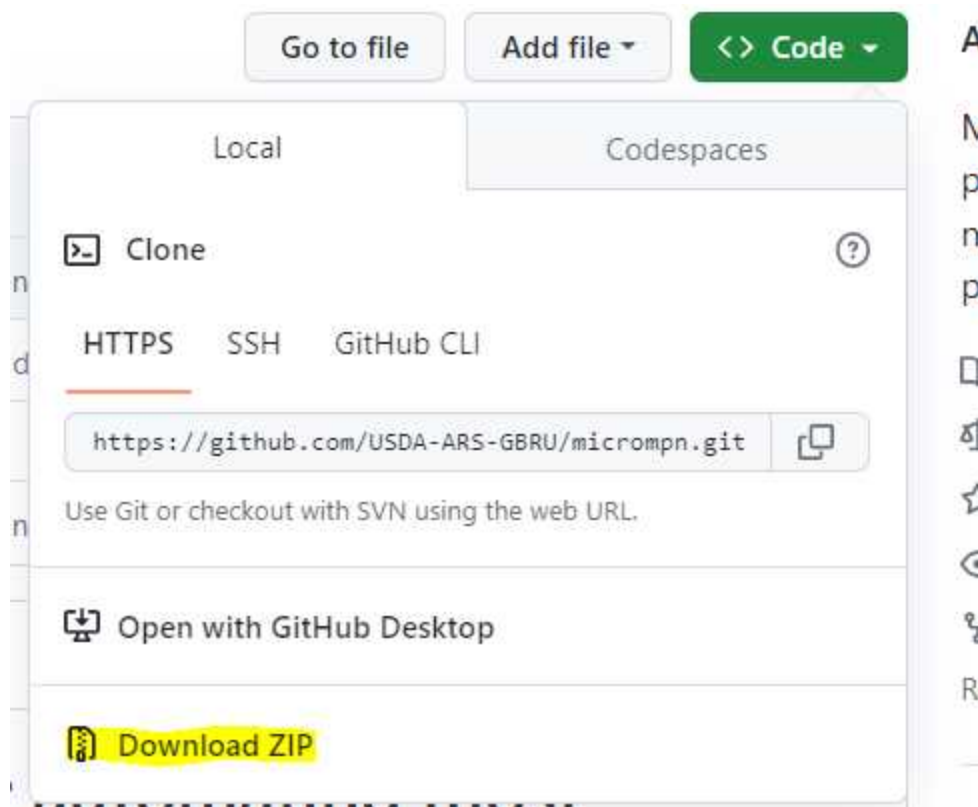
- 3 Step-by-step example on how to run the command-line tool MicroMPN from a Jupyter Notebook.

**** To run micrompn from a Jupyter Notebook use the symbol "!" before micrompn; micrompn was designed to run from the command-line not from a Jupyter Notebook.***

** For ease of documentation and a user-friendly interface, we used Jupyter Notebooks for MPN calculations. For more information on Jupyter Notebooks users are referred to ([Jupyter Project Documentation](#)).*

**For information on how to install packages in a Jupyter Notebook visit [Installing Python Packages from a Jupyter Notebook](#).*

- 3.1 Outside of a notebook, clone or download the MicroMPN repository from GitHub (<https://github.com/USDA-ARS-GBRU/micrompn>). Extract files locally.



- 3.2 Launch a Jupyter Notebook from the Jupyter terminal or directly from the Anaconda Navigator.

Once it launches, your project directory will be displayed in your browser. Make sure the data files needed to run MicroMPN are in your project directory. Then create a new Python notebook in the same directory where all your files are located.

- 3.3 # In a Jupyter Notebook code cell, install MicroMPN by running the following line of code:

```
! pip install C:\Users\location_of_extracted_files\micrompn-main\micrompn-main
```

** In our case, "! pip install" worked because both the shell terminal and kernel are using the same python executable. However, this is not always the case. This is explained in detail in [Installing Python Packages from a Jupyter Notebook](#).*

Ignore this command when re-using the notebook.

- 3.4 # Alternatively, users can install MicroMPN with the following lines of code:

```
import sys
print(sys.executable)
```

Copy the output from "sys.executable", which should be the path to the Python executable active in the kernel.

In a new code cell, run:

```
{sys.executable output} -m pip install C:\Users\location_of_extracted_files\micrompn-main\micrompn-main
```

- 3.5 # In a new code cell, import "micrompn" into the notebook.

```
import micrompn
```

Check proper installation by running:

```
! micrompn --help
```

If installation was successful, user should see the following output:

```
usage: micrompn [-h] --wellmap WELLMAP --data DATA --cutoff CUTOFF --outfile
                OUTFILE [--plate_name PLATE_NAME] [--value_name VALUE_NAME]
                [--well_name WELL_NAME] [--col_name COL_NAME]
                [--row_name ROW_NAME] [--zero_padded] [--trim_positives]
                [--version] [--logfile LOGFILE]

MicroMPN: Software to estimate Most Probable Number (MPN) bacterial abundance
from microplates

optional arguments:
  -h, --help            show this help message and exit
  --wellmap WELLMAP      A TOML file with plate layout specified in wellmap
                        format
  --data DATA           A csv file or a directory containing csv files with
                        the plate name, optical value, and well or row and
                        column data
  --cutoff CUTOFF        The value from the plate reader above which a well is
                        classified as positive
  --outfile OUTFILE      The file path and name for the results
  --plate_name PLATE_NAME
                        The name of the column containing the plate identifier
                        in the data file
  --value_name VALUE_NAME
                        The name of the column containing the optical signal
                        column in the data file
  --well_name WELL_NAME  The name of the column containing the well identifier
                        in the data file
  --col_name COL_NAME    The name of the column containing the plate column
                        identifier in the data file
  --row_name ROW_NAME    The name of the column containing the plate row
                        identifier in the data file
  --zero_padded          If present, the well value in the data file is treated
                        as zero-padded, e.g. A01
  --trim_positives       If present, the list of positive wells will be trimmed
                        to the most dilute all positive dilution and the least
                        dilute all negative dilution. This helps if early
                        dilutions are turbid.
  --version, -v          show program's version number and exit
  --logfile LOGFILE, -l LOGFILE
```

3.6 # To visualize the .toml or .txt file, run the following commands to first install "wellmap" and then import "wellmap" into the notebook:

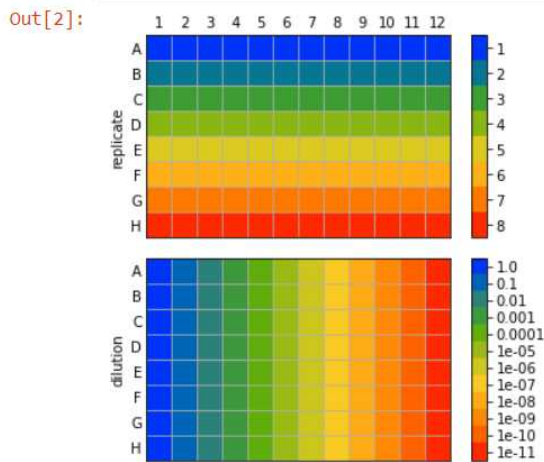
! pip install wellmap #Ignore this command when re-using the notebook.

import wellmap

wellmap.show( microplate.toml)



```
In [2]: wellmap.show("microplate.toml")#prints two versions of the same image (png, img)
```



The Python package "wellmap", which is a dependency of "micrompn", will parse the data from the CSV file based on the .toml or .txt specifications. For more information on how to create a TOML formatted file with different plate layouts visit [wellmap- File format for 96-well plate layouts](#).

- 3.7 # In a new code cell, run "micrompn".
 # CSV file name: input_protocols_io.csv
 # TOML file name: microplate.toml (it could also be a .txt file)

**Make sure the .csv and .toml files are located in the same directory as your Jupyter Notebook (nameofnotebook.ipynb).*

```
# run the following code in a single line
! micrompn --wellmap microplate.toml --data
"C:\location\of\CSV\file\input_protocols_io.csv"
--cutoff 6
--outfile "C:\location\of\CSV\output\file\output_protocols_io.csv"
--plate_name "plate_unique"
--value_name "rfu"
--well_name "plate_well"
--trim_positives
```

- 3.8 Example of output file  output_protocols_io.csv .

	A	B	C	D	E	F	G	H



		plate	sample	mpn	mpn_adj	upper	lower	rarity
	0	plate_3	3	7646785	6885935	18317578	3192197	0.63574

In our experimental setup, ~0.44g of soil were resuspended in 1 mL of volume. Then, 0.1 ml of soil resuspension were transferred to column 1 of a microplate. Since MPN estimated values are based on the volume of a sample used at each dilution step, we must adjust the MPN estimate for the difference in volume by multiplying by 10. The original sample volume in column 1 of our microplate example was 0.1 mL, therefore the concentration of bacteria is MPN/0.1 mL. This is equivalent to a correction of 10 x MPN/1 mL. We would further normalize the MPN estimate by the average mass of soil per well in order to convert MPN/mL to MPN/g. Finally, prior to any statistical analysis, $\log_{10}$ transform MPN values.

Users can avoid this type of correction, if desired, by adjusting the dilution factors to account for the initial volume of soil resuspension.

Additional resources

- 4 We provide users with 96-wells and 384-wells microplate layouts, input files with mock RFU data, MicroMPN output files, and copies of our Jupyter Notebooks in html format.

4.1 Microplate layout: microplate_96_3_samples_vertical_...

96-well plate
3 samples
4 replicates
8 vertical dilutions

input:  96_3_samples_vertical_dilutions.csv

output:  OUTFILE_96_3_samples_vertical_dil...

html:  MicroMPN_96_3_samples_Copy.html

4.2 Microplate layout: microplate_96_2_samples_vertical_...



96-well plate
2 samples
6 replicates
8 vertical dilutions

input:  96_2_samples_vertical_dilutions.csv

output:  OUTFILE_96_2_samples_vertical_dil...

Microplate layout:  microplate_96_2_samples_horizont...

96-well plate
2 samples
4 replicates
12 horizontal dilutions

input:  96_2_samples_horizontal_dilutions....

output:  OUTFILE_96_2_samples_horizontal...

html:  MicroMPN_96_2_samples_Copy.html

4.3 Microplate layout:  microplate_384_4_samples_horizon...

384-well plate
4 samples
8 replicates
12 horizontal dilutions

input:  384_4_samples_horizontal_dilution...

output:  OUTFILE_384_4_samples_horizont...

html:  MicroMPN_384_4_samples_Copy.ht...

4.4 Microplate layout:  microplate_384_6_samples_vertical...



384-well plate
6 samples
8 replicates
8 vertical dilutions

input:  384_6_samples_vertical_dilutions.csv

output:  OUTFILE_384_6_samples_vertical_d...

html:  MicroMPN_384_6_samples_Copy.ht...

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