

# APSCALE: Installation

## Install APSCALE

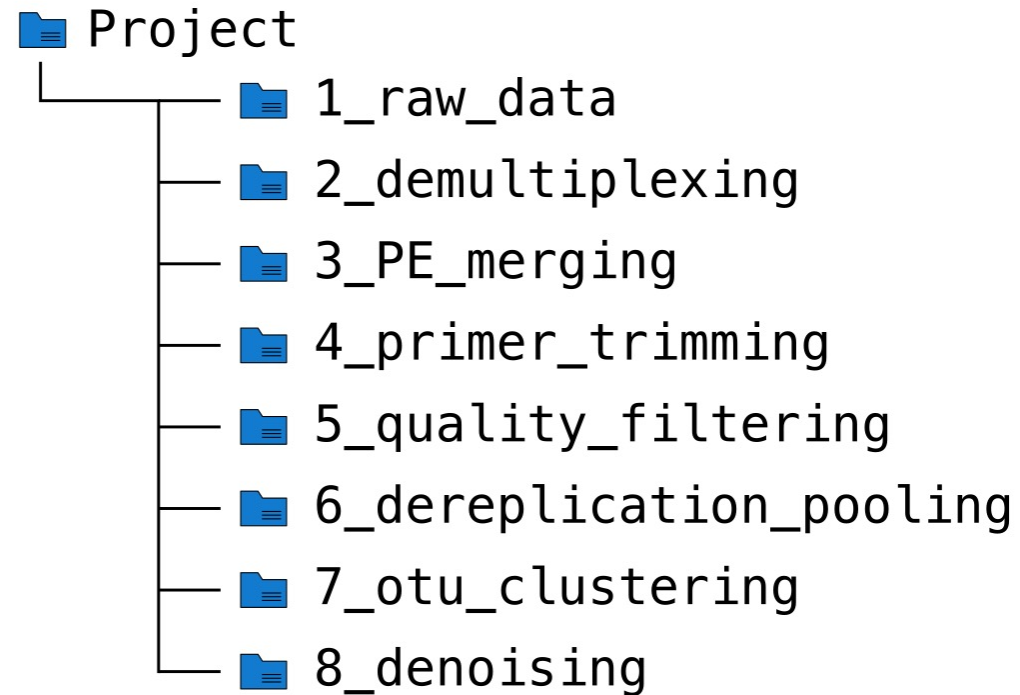
1. Open a terminal (depending on your operating system)
2. Make sure python version 3.7 or higher is installed
3. Type following commands in your terminal (the way python is called may differ on your system):

```
python3.7 -m pip install numpy  
python3.7 -m pip install apscale  
python3.7 -m pip install apscale_gui
```

4. Make sure VSEARCH is installed (click [here](#) for more information):

```
vsearch -version  
>> vsearch v2.20.0_macos_x86_64, 16.0GB RAM, 8 cores
```

# APSCALE: Project management



# APSCALE: Command line

1. Start APSCALE from the terminal:

`apscale`

2. This will print a detailed help message on how to run APSCALE from the command line

3. New projects are created with:

`apscale --create_project tutorial`

4. Alternatively start the APSCALE-GUI as follows

```
tillmacher@TiMa ~ % ascale
usage: ascale [-h] [--create_project NAME] [--run_apscale [PATH]]
              [--pe_merging [PATH]] [--primer_trimming [PATH]]
              [--quality_filtering [PATH]] [--dereplication_pooling [PATH]]
              [--otu_clustering [PATH]] [--denoising [PATH]]

Advanced Pipeline for Simple yet Comprehensive AnaLysEs of DNA metabarcoding
data, see https://github.com/DominikBuchner/apscale for detailed help.

optional arguments:
  -h, --help                show this help message and exit

Creating a project:
  Creates a new ascale project in the current working directory

  --create_project NAME      Creates a new ascale project with the name
                             provided

Running a module:
  Run the ascale pipeline or any specified module. Providing a PATH is
  optional. If no path is provided ascale will run in the current working
  directory.

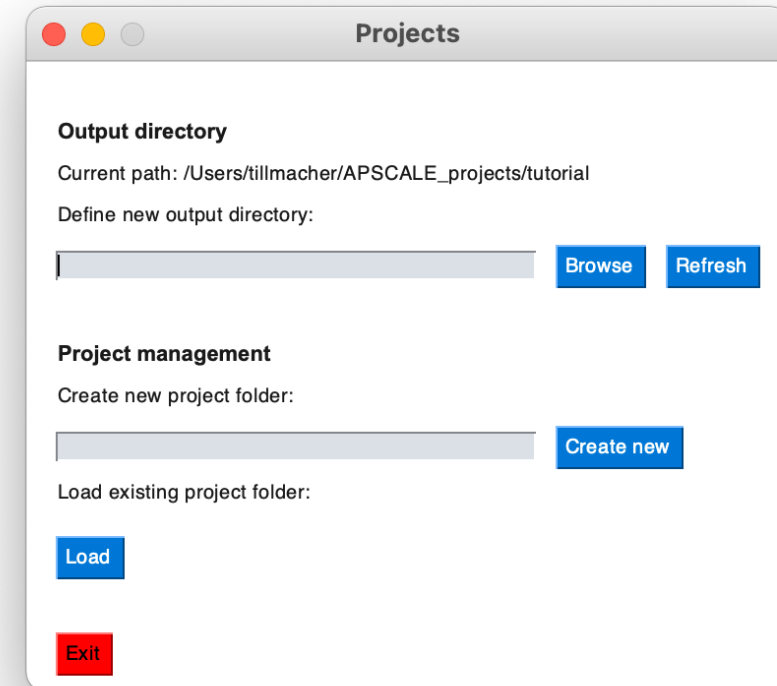
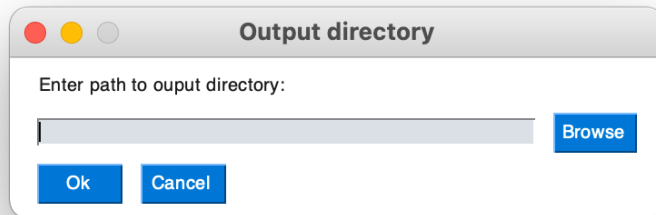
  --run_apscale [PATH]       Run the entire pipeline.
  --pe_merging [PATH]        Run the pe_merging module.
  --primer_trimming [PATH]   Run the primer_trimimng module.
  --quality_filtering [PATH] Run the quality_filtering module.
  --dereplication_pooling [PATH] Run the dereplication_pooling module.
  --otu_clustering [PATH]    Run the otu_clustering module.
  --denoising [PATH]         Run the denoising module.

tillmacher@TiMa ~ %
```

# APSCALE: Graphical user-interface

1. Start APSCALE-GUI from the terminal:

`apscale_gui`



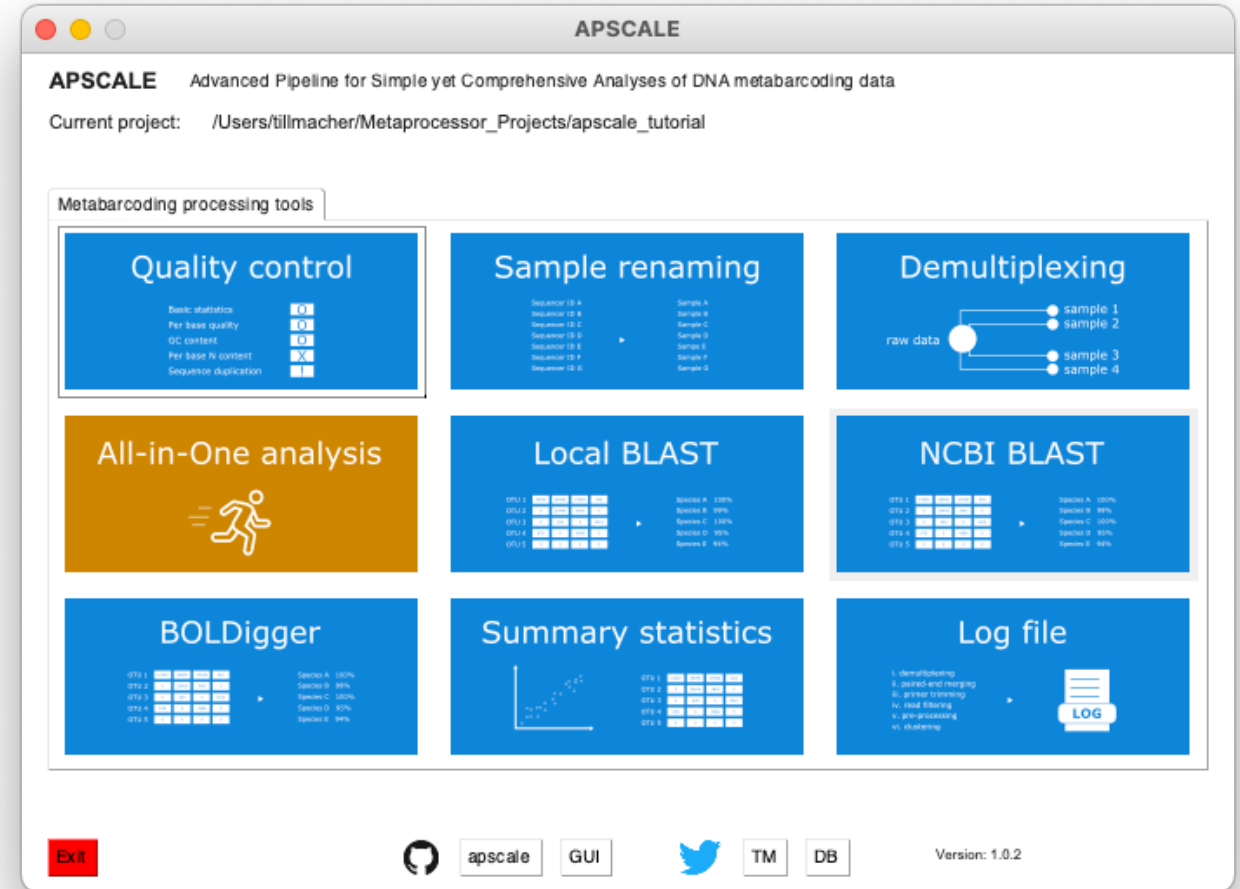
2. Select an APSCALE working directory.
3. All your projects will be saved here

4. Now you can either:
  - create a new project
  - load an existing project

# APSCALE: Graphical user-interface

Various modules are available in the GUI:

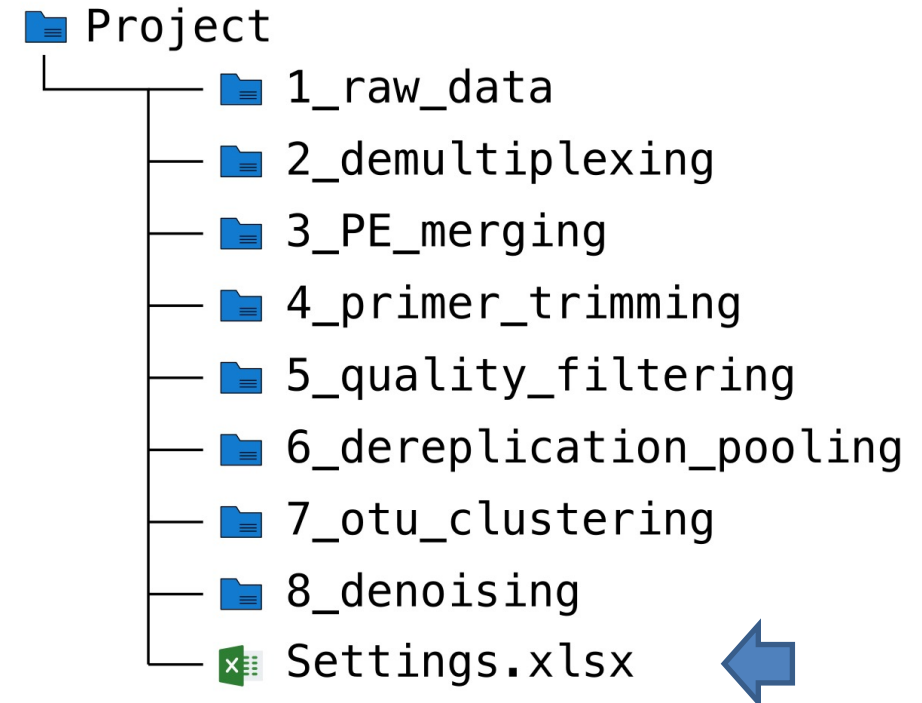
- Quality control
- Sample renaming
- Demultiplexing
- Metabarcoding All-in-One analysis
- Local BLAST
- NCBI BLAST
- BOLDigger
- Summary statistics



# Before you start: Settings

Initially, some settings are required to be specified in the respective Settings.xlsx file of the project:

Setting	Default value
Number of cores	6
Compression level	6
maxdiffpct	25
maxdiff	199
minovlen	5
P5 Primer (5' - 3')	
P7 Primer (5' - 3')	
Anchoring	False
maxEE	1
min length	
max length	
min size to pool	5
pct id	97
alpha	2
minsize	8



# Before you start: Settings

Settings can either be changed by:

- Opening the Settings.xlsx in Excel.
  - Starting the All-in-One analysis in the GUI, entering the new settings and applying them to the Excel file.
- 
- APSCALE will automatically read your settings from the Settings.xlsx when starting the data processing.
  - Detailed information on how to choose the settings are explained later in the tutorial.

The screenshot shows the 'All-in-One analysis' GUI window. It contains several sections for configuring the analysis:

- 1. Modify settings:** Includes a text box 'Load settings file and modify as required.' and two buttons: 'Open settings file' (blue) and 'Apply new settings' (green).
- 2. Paired-end merging:** A checked checkbox followed by three input fields: 'maxdiffpct: 25', 'maxdiffs: 199', and 'minovlen: 5'.
- 3. Primer trimming:** A checked checkbox followed by two text boxes for primers: 'P5 Primer (5' - 3'): AAACCTCGTGCCAGCCAC' and 'P7 Primer (5' - 3'): CAAACTGGGATTAGATAC', and a dropdown for 'Anchoring: False'.
- 4. Quality filtering:** A checked checkbox followed by three input fields: 'maxEE: 1', 'min length: 120', and 'max length: 220'.
- 5. Dereplication & pooling:** A checked checkbox followed by an input field: 'min size: 2'.
- 6.1 OTU clustering:** A checked checkbox followed by an input field: 'pct id: 97'.
- 6.2 Denoising:** A checked checkbox followed by two input fields: 'alpha: 2' and 'min size: 8'.
- Data clean-up:** An unchecked checkbox followed by a text box: 'Remove all temporary data to save storage space.'

At the bottom, there is a blue 'Run analysis' button, a checked checkbox for 'Minimize APSCALE', and a red 'Exit' button.