Analysis of EPI2ME 16S CSV Output

Expected Duration: 10 minutes

The EPI2ME 16S (and WIMP) analyses allow the download of a summary table of the results. However this table does not contain full lineage information and so it is not immediately useful to create the Sankey tree diagrams that EPI2ME displays in its web interface.

The following short code fragments, demonstrate how to decorate the EPI2ME data table and aggregate the read counts.

An example 16S report can be found here.

Computational requirements for this tutorial:

- A computer running the **EPI2ME Labs** server
- 8Gb RAM

Getting started

Before anything else we will create and set a working directory:

In [10]:
```
from epi2melabs import ping
tutorial_name = "16s_lineage"
pinger = ping.Pingu()

# create a work directory and move into it
working_dir = '/epi2melabs/{}/'.format(tutorial_name)
!mkdir -p "$working_dir"
%cd "$working_dir"
```

```
/epi2melabs/16s_lineage
```

Install additional software

In order to decorate the EPI2ME results file with lineage information we will use the taxonkit tool. The codebox below will download this tool and also `csvkit` which we will use to convert the EPI2ME file from a comma-separated file to a tab-seperated file (which taxonkit requires)

Please note that the software installed is not persistent and this step will need to be re-run if you stop and restart the EPI2ME Labs server.

In [ ]:
```
!pip install csvkit
!wget https://github.com/shenwei356/taxonkit/releases/download/v0.6.0/taxonkit_linux_amd64.tar.gz
!tar -xzvf taxonkit_linux_amd64.tar.gz
```

Taxonkit requires the NCBI taxonomy database to function, let's download and decompress that:

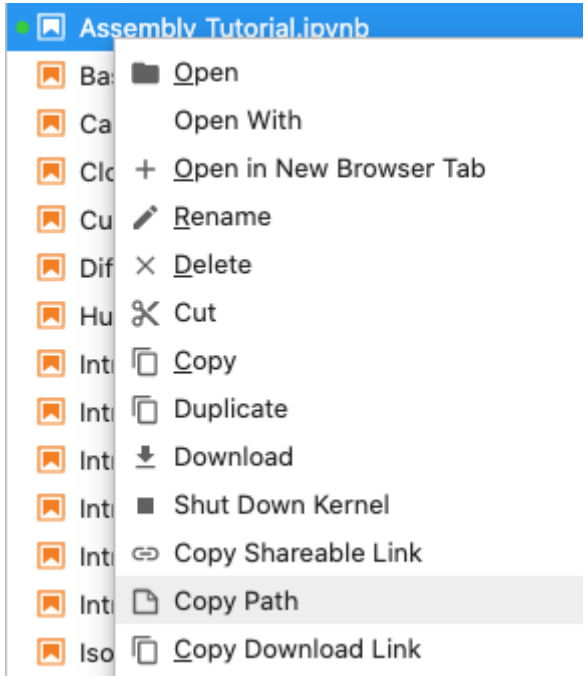
In [ ]:
```
!mkdir -p "$working_dir"/taxdump
%cd "$working_dir/taxdump"
!wget http://ftp.ncbi.nih.gov/pub/taxonomy/taxdump.tar.gz
!tar -xzvf taxdump.tar.gz
%cd "$working_dir"
```

We now have all we need to get going:

- the EPI2ME results
- taxonkit
- The NCBI taxonomy

Using your own data

If you wish to analyse your own data rather than the sample data, you can edit the value `.fastq input` variable below. To find the correct full path of a directory you can navigate to it in the `Files` browser to the left-hand side, right-click on the file and select `Copy path`:



The location shared with the EPI2ME labs server from your computer will show as `/epi2melabs`, for example a file located at `/data/my_gridion_run/fastq_pass` on your computer will appear as `/epi2melabs/my_gridion_run/fastq_pass` when it is the `/data` folder that is shared.

Data Entry

Please select to use the sample data, or enter your own.

In [1]:
```
import os
from epi2melabs.notebook import InputForm, InputSpec

import ipywidgets as widgets

use_sample_data = None
epi2me_results_file = None
annotated_output = None

def process_form(inputs):
```

```
    global use_sample_data
    global epi2me_results_file
    global annotated_output
    use_sample_data = inputs.use_sample_data
    epi2me_results_file = os.path.abspath(inputs.epi2me_results_file)
    annotated_output = inputs.annotated_output

    if use_sample_data:
        print("Using sample data")
        bucket = "ont-exd-int-s3-euwst1-epi2me-labs"
        domain = "s3-eu-west-1.amazonaws.com"
        site = "https://{}.{}".format(bucket, domain)
        fname = "242318_classification_16s_barcode-v1.csv"

        print("Downloading sample data...")
        !wget -q $site/misc/$fname \
            && echo "Download complete" || echo "Download failed"
        print("Done.")
        epi2me_results_file = os.path.abspath(fname)

    if not os.path.isfile(epi2me_results_file):
        print("Input file does not exist")
    print("\nFirst 10 lines of file:")
    !head "$epi2me_results_file" || echo "File not readable"

inputs = InputForm(
    InputSpec('use_sample_data', 'Use sample data', True),
    InputSpec('epi2me_results_file', 'EPI2ME Results File', ''),
    InputSpec('annotated_output', 'Output filename',
              '/epi2melabs/16s_lineage/epi2me_results_with_lineage.csv'))
inputs.add_process_button(process_form)
inputs.display()
```

```
VBox(children=(HBox(children=(Label(value='Use sample data', layout=Layout(width='150px')), interactive(childr…
```

After entering your inputs above and pressing `Enter`, select `Run selected Cell and All Below` from the `Run` menu at the top of the page. If you need to analyse a second file just come back here, change `epi2me_results_file` to a new value and then select `Runtime > Run after`.

Analysis

Let's first write a code function to read in and perform several conversions on the EPI2ME file. If you would like to use the methods of this notebook in your own code, this code cell is about all you will need:

In [19]:
```
# Code for annotating an EPI2ME results file with lineage information (click play)
import pandas as pd
import os
def parse_epi2me(fname):
    dirname = os.path.dirname(fname)
    tsvtmp = os.path.join(dirname, "epi2me2lineage.tsv")
    lintmp = os.path.join(dirname, "epi2me2lineage.lin.tmp")
    fmttmp = os.path.join(dirname, "epi2me2lineage.fmt.tmp")

    print("Converting to TSV")
    !csvformat -T "$fname" > "$tsvtmp"
    # need to know the position of the "taxid" column and the number of columns
    try:
        with open(tsvtmp, 'r') as fh:
            header = fh.readline().rstrip().split()
            taxid_col = 1 + header.index('taxid')
            lineage_col = 1 + len(header)  # this will be added in
    except:
        raise IndexError("Could not find 'taxid' column in input.")
    print("Running lineage")
    !"$working_dir/taxonkit" lineage --data-dir taxdump "$tsvtmp" -i "$taxid_col" 2>/dev/null > "$lintmp"
    print("Running reformat")
    !"$working_dir/taxonkit" reformat --data-dir taxdump -i "$lineage_col" "$lintmp" 2>/dev/null > "$fmttmp"
    print("Munging data")
    epi2me = pd.read_csv(fmttmp, sep='\t')
    # rename some columns so they don't clash with the lineage info
    epi2me.columns = epi2me.columns[0:len(epi2me.columns)-2].to_list() + ['_lineage', 'lineage']
    epi2me = epi2me.rename(columns={'species': 'species_name', 'genus':'genus_name'})
    # extract the lineage info into its on columns in the table
    lineage = epi2me['lineage'].str.split(";", expand=True)
    lineage.columns = ['kingdom', 'phylum', 'class', 'order', 'family','genus', 'species']
    epi2me = pd.concat((epi2me, lineage), axis=1)
    !rm -rf "$tsvtmp" "$lintmp" "$fmttmp"
    return epi2me
```

We can now read the input file, annotate it with the lineage information, and write out the annotated table.

In [13]:
```
epi2me = parse_epi2me(epi2me_results_file)
epi2me.to_csv(annotated_output)
pinger.send_notebook_ping('stop', tutorial_name)
print("Annotated data table written to: {}. Download from the filebrowser.".format(annotated_output))
```

```
Converting to TSV
Running lineage
Running reformat
Munging data
Annotated data table written to: /epi2melabs/16s_lineage/epi2me_results_with_lineage.csv. Download from the filebrowser.
```

The output table is the same as the input EPI2ME results file with additional columns indicating the taxonomic ranks to with each read has been assigned. For example, here is the start of the table:

In [14]:
```
epi2me = pd.read_csv(annotated_output)
display(epi2me.head())
```

	Unnamed: 0	readid	runid	barcode	exit_status	taxid	species_taxid	species_name	accuracy	genus_tax
0	0	0003c930-069c-4c63-a8df-1a0ca50992c4	711db73c212e422fad11ca3c0ed596fc	NaN	Classification successful	404937.0	404937.0	Anoxybacillus thermarum	93.88	150247
1	1	000d4674-7d99-4fed-8ba6-ab5781ab95b7	711db73c212e422fad11ca3c0ed596fc	NaN	Classification successful	43657.0	43657.0	Pseudoalteromonas luteoviolacea	93.37	53246
2	2	00248183-faee-4f5b-a9fe-feb8e241db92	711db73c212e422fad11ca3c0ed596fc	NaN	Classification successful	1855725.0	1855725.0	Mucilaginibacter antarcticus	95.50	423349
3	3	0029656e-5028-48e5-8822-0ba770d9ccf6	711db73c212e422fad11ca3c0ed596fc	NaN	Classification successful	878213.0	878213.0	Actinomycetospora iriomotensis	92.25	402649
4	4	002b9804-6cd0-42cd-b86e-6c71cf01bf4e	711db73c212e422fad11ca3c0ed596fc	NaN	Classification successful	2027860.0	2027860.0	Mucilaginibacter rubeus	93.07	423349

5 rows × 21 columns

With this data table we can now extract counts of reads at any of the taxonomic ranks, for example:

In [3]:
```
def process_form(inputs):
    def replace_empty(index):
        try:
            idx = index.index('')
        except ValueError:
            pass
        else:
            index[idx] = 'Unknown'
        return index
```

```
    if not inputs.split_by_barcode:
        results = (
            epi2me[inputs.aggregation_rank]
            .value_counts()
            .reset_index()
            .rename(columns={'index':inputs.aggregation_rank, inputs.aggregation_rank:'count'}))
        results[inputs.aggregation_rank] = replace_empty(results[inputs.aggregation_rank].tolist())
        results.sort_values('count', ascending=False, inplace=True)
    else:
        results = (
            epi2me[[inputs.aggregation_rank, 'barcode']]
            .groupby([inputs.aggregation_rank, 'barcode'])
            .size().unstack(fill_value=0))
        results['total'] = results.sum(axis=1)
        results.sort_values('total', ascending=False, inplace=True)
        results.index = replace_empty(results.index.tolist())
    print("Top 10 groups.")
    display(results.head(10))
    output = 'aggregated_counts_by_{}.csv'.format(inputs.aggregation_rank)
    results.to_csv(output)
    print("Results written to {}, download from the filebrowser.".format(output))

outputs = InputForm(
    InputSpec('aggregation_rank', 'Taxonomic Rank', ['kingdom', 'phylum', 'class', 'order', 'family','genus', 'species']),
    InputSpec('split_by_barcode', 'Split barcodes', False))
outputs.add_process_button(process_form)
outputs.display()
```

```
VBox(children=(HBox(children=(Label(value='Taxonomic Rank', layout=Layout(width='150px')), interactive(childre…
```

Some notes

The EPI2ME table provides a `taxid`, `species_taxid` and a `genus_taxid`. EPI2ME provides some sanity checking on its classification. If the top hits of a read are from different genera the `taxid` will be empty, that is to say the read is "Unclassified". The code above is using the value of the `taxid` field to derive the lineage information.