**Analysis of microbiome nanopore sequence data**

1. Upload data to Galaxy.

Create an Galaxy account and log in at <https://nanopore.usegalaxy.eu>. Click ***Upload Data*** icon, choose ***Collection*** tab on the top. Use Collection because in the Nanopore sequencing output, a single sample usually contains multiple read files. Then select ***Choose local files*** and browse to the nanopore data in the local computer. Choose *fastq* for the file type. It’s important not to choose *Auto-detect*.

2. Concatenate data.

In Galaxy left side bar, choose ***Concatenate datasets*** and choose the data collection uploaded from step 1, and click execute.

3. Taxon identification in Kraken 2.

In Galaxy left side bar, choose ***Kraken 2***. Select *Single Read*. Choose the concatenated data as input. Chose confidence level at 0.1. Under Create Report, choose *Print a report with aggregrate counts/clade to file*, and *Report counts for ALL taxa, even if counts are zero, but*

disable *Kraken 1's kraken-mpa-report*. Finally, choose **SILVA 138 16S (2021)** database, and click execute. When finished, the report should contain six columns:

Col 1. Percentage of fragments covered by the clade rooted at this taxon

Col 2. Number of fragments covered by the clade rooted at this taxon

Col 3. Number of fragments assigned directly to this taxon

Col 4. A rank code

Col 5. NCBI taxonomic ID number

Col 6. Indented scientific name

4. Compare samples in Pavian (<https://github.com/fbreitwieser/pavian>)

Pavian is a R package. To install Pavian in R, type the following commands.

Text

Description automatically generated

To run Pavian from R, type



It will launch a Shiny app and the Kranken 2 reports can be uploaded. To display an OUT table, click **comparison** in the left side bar. Then Choose a taxonomic rank (green tab) and select one or multiple desired comparison criteria (blue tab). Download the full table in a tab-separated value format for data visualization.

5. Data visualization in R.

Use the OTU table produced by Pavian to generate a simple heatmap in R using the default *heatmap()* function.