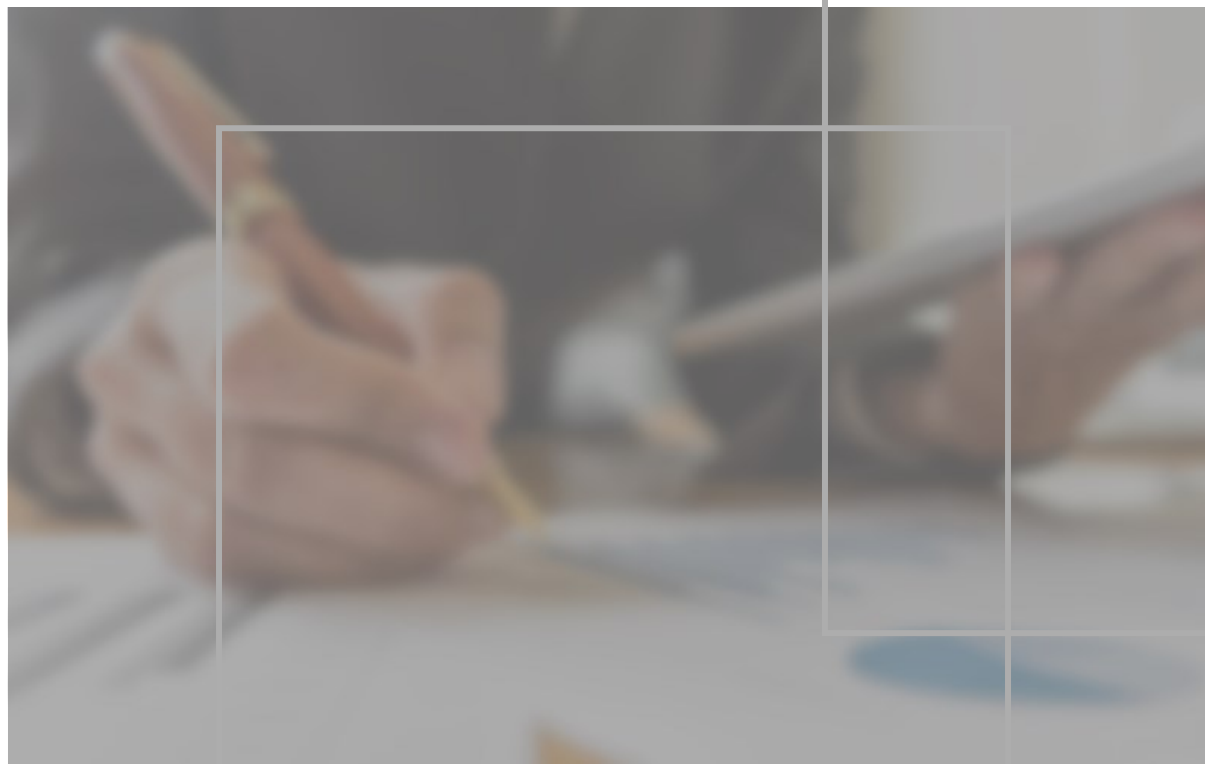




Report Generation

Advanced Molecular Detection
Southeast Region Bioinformatics



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Updates



- In September we'll be scheduling our next check-in calls
- Next presentations:
 - 09/15- Legionella Tools
 - 09/29- Neissflow

Overview

Purpose

- This pipeline tool is developed by the Florida BPHL team to streamline and generate genomic epidemiology reports which consist of data preprocessing and report generation of phylogenetic tree, 3D plots, generation of AMR genes, and Geomap plot.

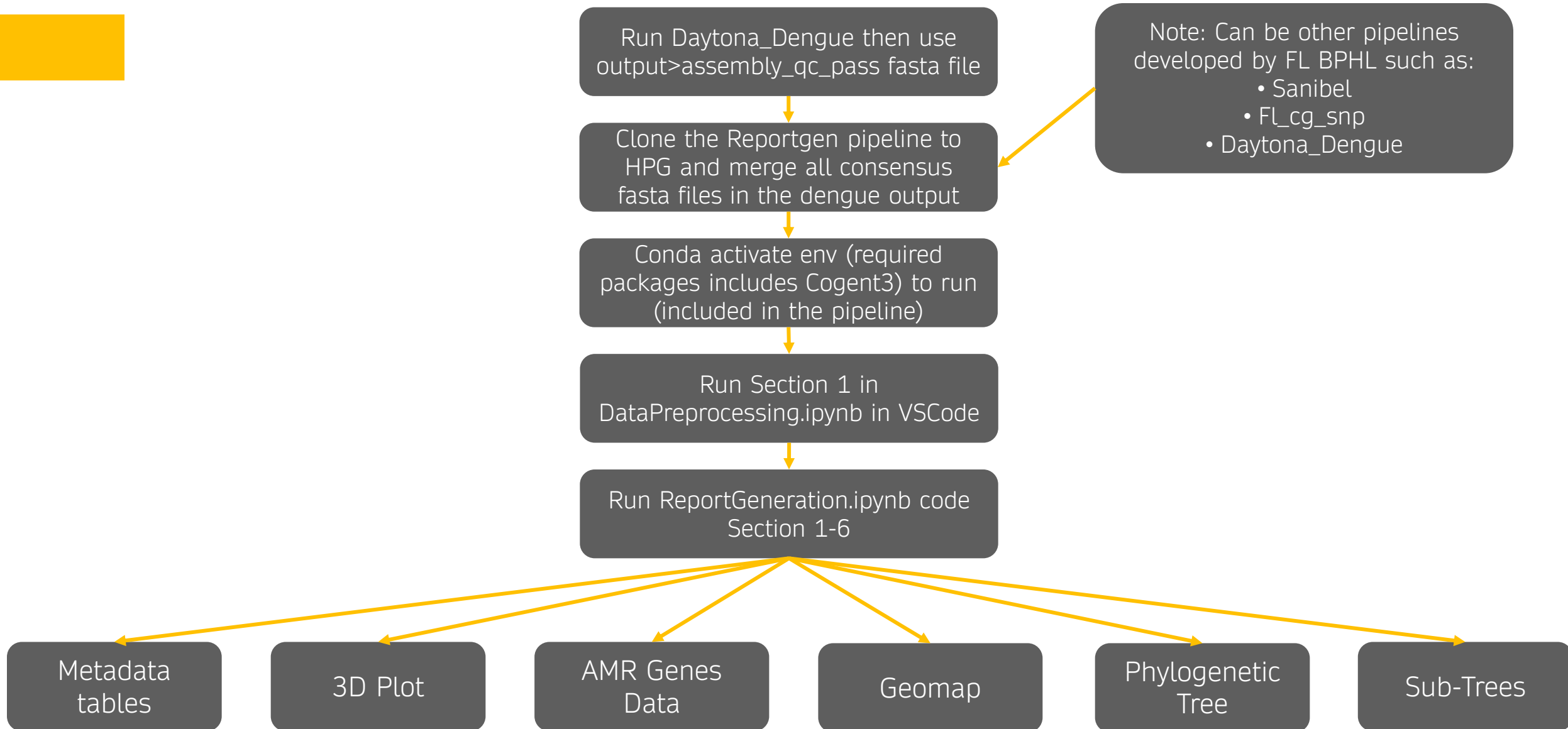
Usage

- Can be used by public health labs, researchers, and sequencing centers to generate standardized epidemiology reports for surveillance and research purposes only.

Dependencies

- Conda; Pandas, Plotly, Bio, Matplotlib, Numpy, Sklearn, Warnings, Json
- VScode
- Cogent3

Workflow



Application



Objective

Use a Dengue_Daytona output dataset and generate a genomic epidemiology report from the Report Generation pipeline.

Application Cont.

1. BPHL-Molecular Github:

<https://github.com/BPHL-Molecular/ReportGen>

2. Clone the Code and copy the Data to your Analysis Directory

3. Create an environment in conda using the environmental.yml file that is provided:

```
conda env create --name ReportGen -f environment.yml
```

4. Run HPG on VS Code:

Note: Review VS Code Office Hours on connecting HPG to VS Code

5. Install all the Required Packages in the ReportGen Env:

Cogent3, Pandas, Plotly, Bio, Matplotlib, Numpy, Sklearn, Warnings, Json

Application Cont.

Run Daytona_Dengue

Note: Review Previous Office Hours on How to Run Daytona_Dengue

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/2024/Daytona_dengue1212/

Name

- ..
- configs
- fastqs
- modules
- output
- primers
- reference
- work
- daytona_dengue.52626148.err
- daytona_dengue.52626148.out
- daytona_dengue.nf
- daytona_dengue.sh
- kraken2_viral.sh
- LICENSE
- nextflow.config
- params.yaml
- README.md
- rename_aqp.py
- renamefile.sh
- table.py

Application Cont.

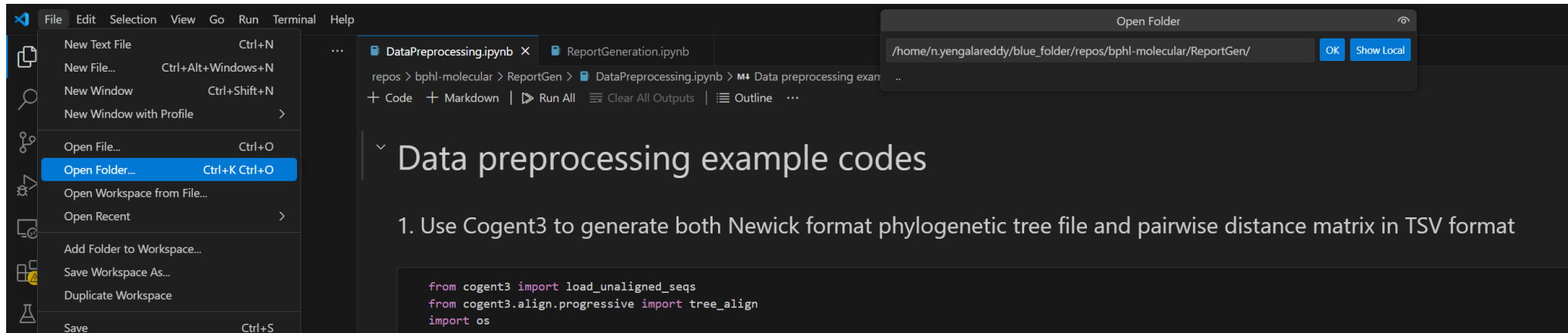
```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/2024/Daytona_dengue1212/  
Name  
└─ ..  
└─ configs  
└─ fastqs  
└─ modules  
└─ output  
└─ primers  
└─ reference  
└─ work  
└─ daytona_dengue.52626148.err  
└─ daytona_dengue.52626148.out  
└─ daytona_dengue.nf  
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└─ kraken2_viral.sh  
└─ LICENSE  
└─ nextflow.config  
└─ params.yaml  
└─ README.md  
└─ rename_aqp.py  
└─ renamefile.sh  
└─ table.py
```

cat *.fa > merged.consensus.fa
Note: Run this command in the
assembly_qc_pass directory

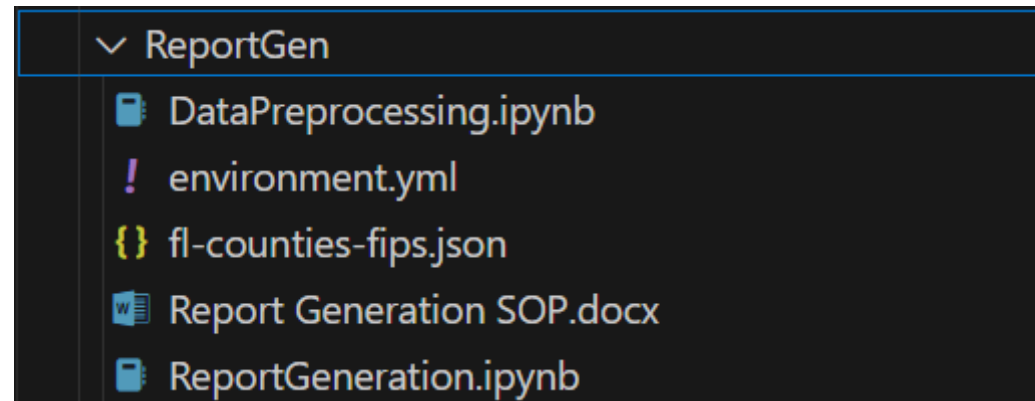
```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/2024/Daytona_dengue1212/output/  
Name  
└─ ..  
└─ alignment  
└─ assembly  
└─ assembly_qc_pass  
└─ bbduk  
└─ fastqc  
└─ fastqc_clean  
└─ humanscrubber  
└─ kraken_out_broad  
└─ multiqc  
└─ report  
└─ variant  
└─ variant_qc_pass
```

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/2024/Daytona_dengue1212/output/assembly_qc_pass/  
Name  
└─ ..  
└─ SER_del  
└─ J137.consensus.fa  
└─ J138.consensus.fa  
└─ J196.consensus.fa  
└─ J198.consensus.fa  
└─ J210.consensus.fa  
└─ J248.consensus.fa  
└─ J320.consensus.fa  
└─ J339.consensus.fa  
└─ J357.consensus.fa  
└─ J369.consensus.fa  
└─ merged.consensus.fa  
└─ T91.consensus.fa  
└─ T93.consensus.fa  
└─ T94.consensus.fa  
└─ T107.consensus.fa
```

Application Cont.



Application Cont.



Application Cont.

```
repos > bphi-molecular > ReportGen > DataPreprocessing.ipynb > Data preprocessing example codes
+ Code + Markdown | ▶ Run All ⏸ Restart ≡ Clear All Outputs | 📄 Jupyter Variables ≡ Outline ...
```

coagent3 (Python 3.11.13)

▼ Data preprocessing example codes

▼ 1. Use Cogent3 to generate both Newick format phylogenetic tree file and pairwise distance matrix in TSV format

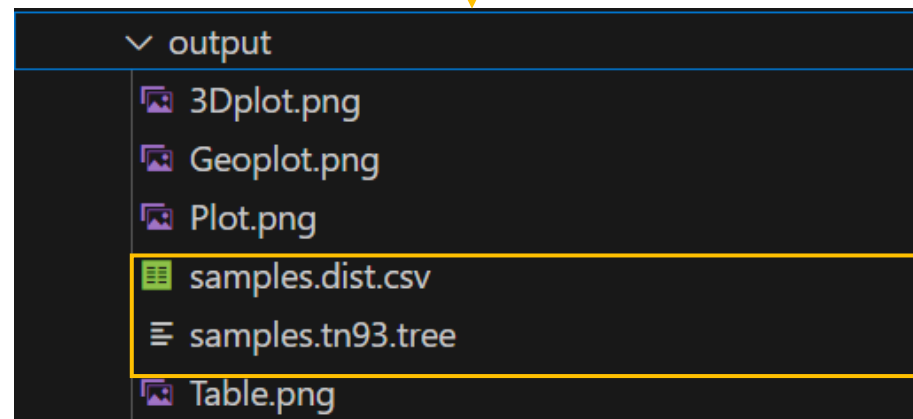
```
from cogent3 import load_unaligned_seqs
from cogent3.align.progressive import tree_align
import os

consensus_fasta_file = "merged.consensus.fasta" # Replace with your file path
tree_newick_file = "samples.tn93.tree" # Replace with your file path
dists_csv_file = "samples.dist.csv" # Replace with your file path

seqs = load_unaligned_seqs(consensus_fasta_file, moltype="dna")
aln, tree = tree_align("TN93", seqs, show_progress=False)
tree.write(tree_newick_file)

dists = aln.distance_matrix(calc="tn93", show_progress=False)
dists_df = dists.to_table().to_dataframe()
dists_df.to_csv(dists_csv_file)
```

[] Python



Application Cont.

DataPreprocessing.ipynb • ReportGeneration.ipynb •

repos > bphi-molecular > ReportGen > ReportGeneration.ipynb > M4 Report generation example codes > M4 1. Show the metadata as a table

+ Code + Markdown ▶ Run All ⌂ Restart ☰ Clear All Outputs 📄 Jupyter Variables 📖 Outline ...

cogent3 (Python 3.11.13)

Report generation example codes

1. Show the metadata as a table

```
import pandas as pd
import plotly.graph_objects as go
import os

## -----
## Load the meta data, in csv, tsv, or excel format -
## -----
meta_file = "meta.csv" # Replace with your file path
data = pd.read_csv(meta_file)
```

Sample ID	Collection Date	County of Origin	speciesID_kraken	speciesID_mash	NDM
J196	2023-02-02	Pinellas	Enterobacter cloacae	Enterobacter cloacae	blaNDM-7
J248	2023-02-10	Martin	Enterobacter hormaechei	Enterobacter cloacae	blaNDM-7
J369	2023-03-01	Alachua	Enterobacter cloacae	Enterobacter cloacae	blaNDM-7
T94	2023-03-06	Pinellas	Enterobacter hormaechei	Enterobacter cloacae	blaNDM-7
J138	2023-01-18	Orange	Escherichia coli	Escherichia coli	blaNDM-5
J357	2023-02-24	Martin	Escherichia coli	Escherichia coli	blaNDM-5
J320	2023-02-17	Orange	Klebsiella aerogenes	Klebsiella aerogenes	blaNDM-7
J314	2023-02-14	Alachua	Klebsiella michiganensis	Klebsiella michiganensis	blaNDM-1
J137	2023-01-15	Alachua	Klebsiella pneumoniae	Klebsiella pneumoniae	blaNDM-1
J210	2023-02-08	Hillsborough	Klebsiella pneumoniae	Klebsiella pneumoniae	blaNDM-1
J339	2023-02-20	Duval	Klebsiella pneumoniae	Klebsiella pneumoniae	blaNDM-1
T91	2023-03-04	Orange	Klebsiella pneumoniae	Klebsiella pneumoniae	blaNDM-1
T93	2023-03-05	Duval	Klebsiella pneumoniae	Klebsiella pneumoniae	blaNDM-1
J198	2023-02-06	Duval	Klebsiella variicola	Klebsiella variicola	blaNDM-1
T107	2023-03-10	Alachua	Klebsiella variicola	Klebsiella variicola	blaNDM-1

Application Cont.



```
repos > bphl-molecular > ReportGen > ReportGeneration.ipynb > 1. Show the metadata as a table
+ Code + Markdown | ▶ Run All ↺ Restart ☰ Clear All Outputs | Jupyter Variables ☰ Outline ... cogent3 (Python 3.11.13)
```

2. Plot phylogenetic tree as a whole tree given a newick format file

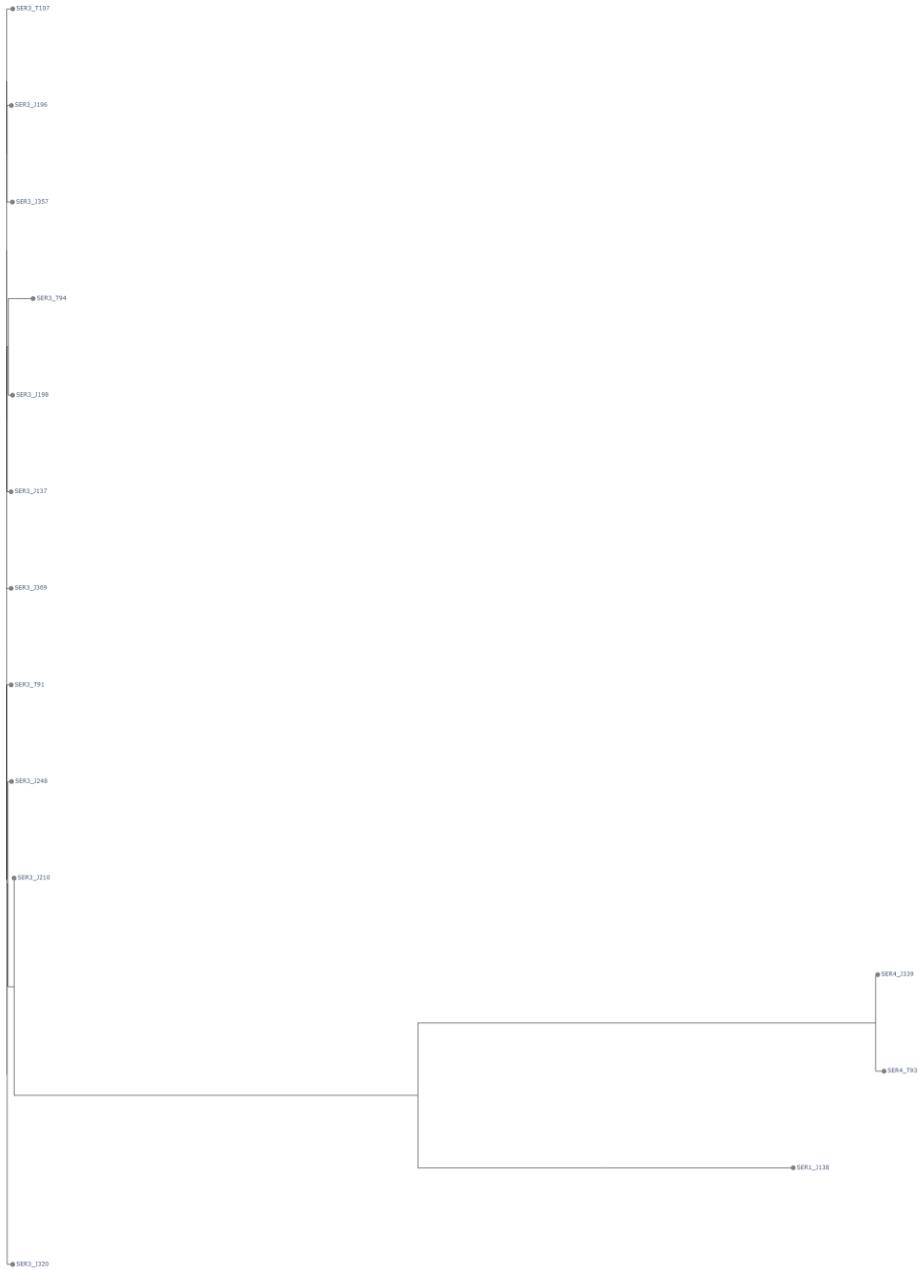
```
from Bio import Phylo
import pandas as pd
from plotly.offline import init_notebook_mode, iplot
import plotly.graph_objects as go

init_notebook_mode(connected=False)
import colorsys
import numpy as np
import os
import random
```

Application Cont.



Phylogeny of *NDM* Positive CRDs samples
14 samples



Application Cont.



repos > bphl-molecular > ReportGen > ReportGeneration.ipynb > Report generation example codes > 2. Plot phylogenetic tree as a whole tree given a newick format file > from Bio import Phylo

+ Code + Markdown | ▶ Run All ↺ Restart ⌵ Clear All Outputs | 📄 Jupyter Variables 📖 Outline ... cogent3 (Python 3.11.13)

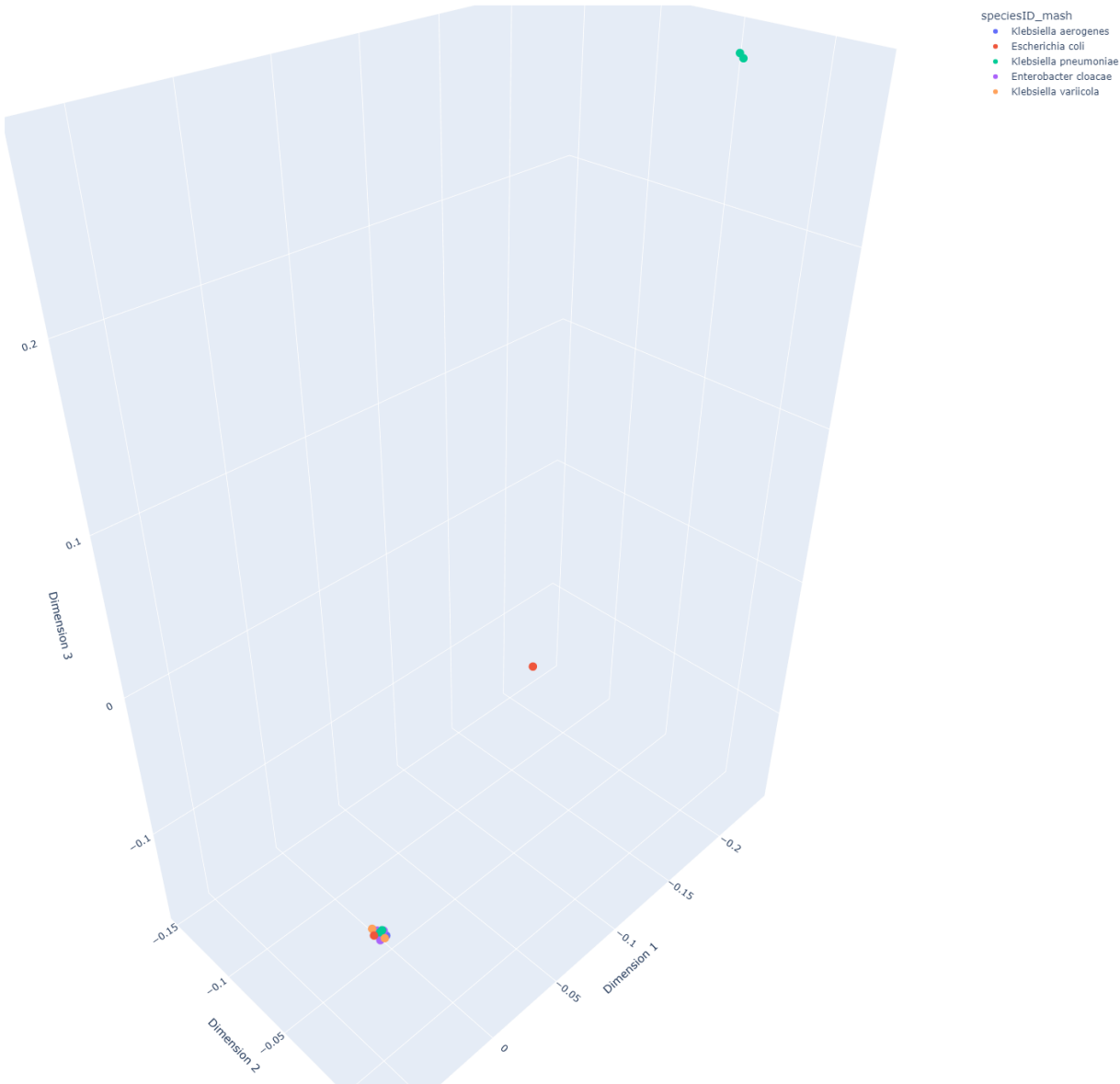
4. 3D plot of the samples based on the pairwise distances. Either Multidimensional Scaling (MDS) or Uniform Manifold Approximation and Projection (UMAP) may be used for generating the coordinates.

```
import pandas as pd
import numpy as np
from sklearn.manifold import MDS
import matplotlib.pyplot as plt
import seaborn as sns
import plotly.express as px
import umap.umap_ as umap
from sklearn.decomposition import PCA
import warnings
```


Application Cont.



3D MDS Plot Colored by speciesID_mash



Application Cont.

```
repos > bphl-molecular > ReportGen > ReportGeneration.ipynb > Report generation example codes > 2. Plot phylogenetic tree as a whole tree given a newick format file > from Bio import Phylo
```

+ Code + Markdown | ▶ Run All ↺ Restart ≡ Clear All Outputs | Jupyter Variables ≡ Outline ... cogent3 (Python 3.11.13)

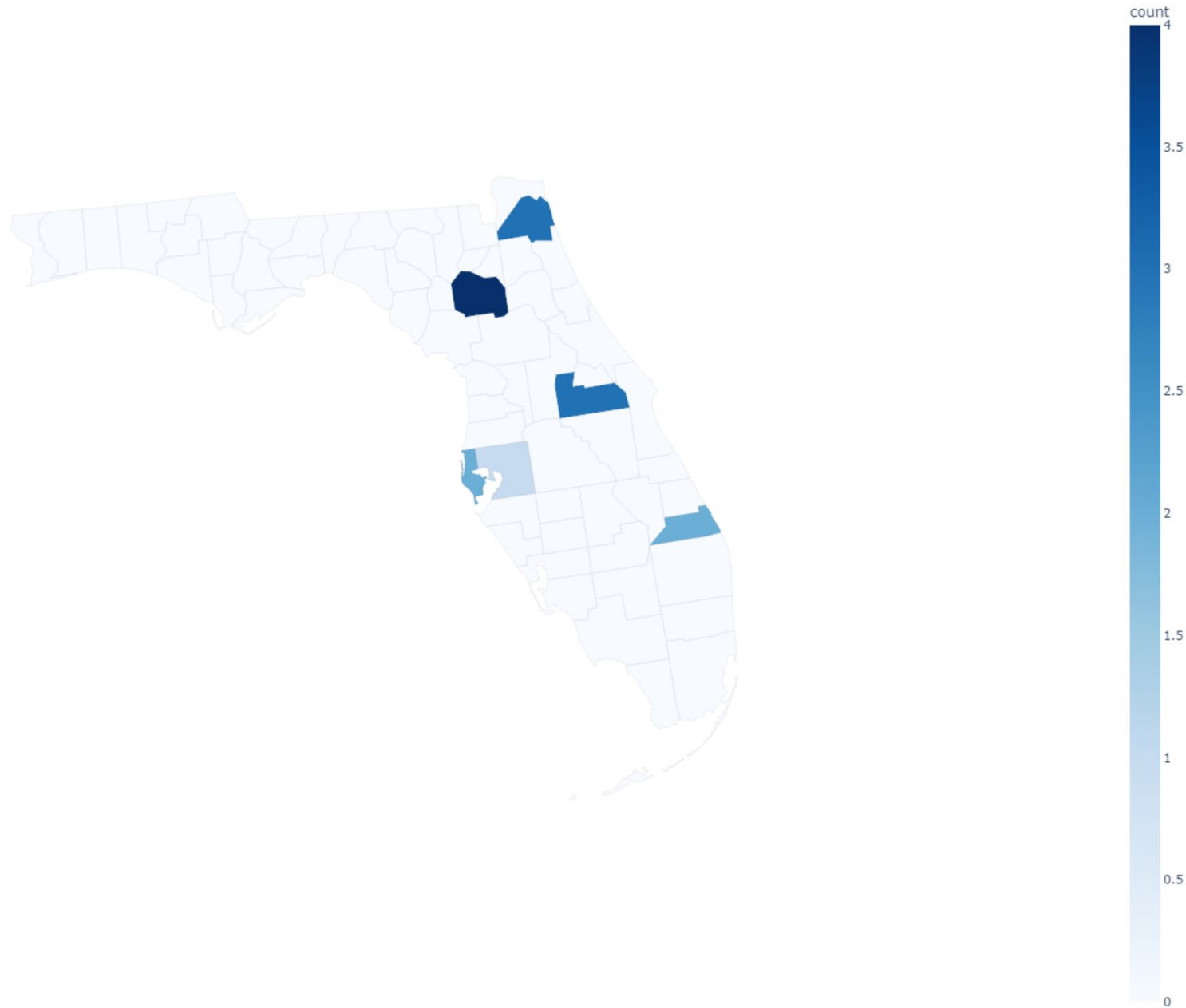
6. Geo map by county

```
> ~
import plotly.graph_objects as go
import pandas as pd
import plotly.express as px
import os
import json

# Load the geojson file
cwd = os.getcwd()
with open("../Data/meta/fl-counties-fips.json", "r") as f: # update the path to the geojson file
    geojson = json.load(f)

# Load the CSV file
meta = pd.read_csv("../Data/meta/meta_data.csv") # example meta data file; you may change the meta data file here
renamed_columns = ["Sample ID", "Collection Date", "County"] # only show data for these columns; you may change the column names here
meta.columns = renamed_columns
```

Application Cont.



Conclusion

- ✓ Fundamentals of Report Generation
- ✓ Installation and setup of Report Generation in HPG
- ✓ Successfully executed job query for Report Generation
- ✓ Generated output files



Advanced Molecular Detection

Southeast Region Bioinformatics

Questions?

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