05 Jan 2026

mashwrapper

Advanced Molecular Detection

Southeast Region Bioinformatics

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Overview

Purpose

- This Nextflow pipeline uses Mash-based k-mer comparisons to rapidly screen sequencing reads against reference genomes to identify the most likely organism, such as SARS-CoV-2 and/or *Legionella*, from raw sequencing data.

Usage

- It enables public health laboratories to quickly confirm organism identity, validate sample content, and support downstream workflows such as lineage assignment, phylogenetic analysis, and genomic surveillance across multiple samples.

Dependencies

- Nextflow and Apptainer/Singularity

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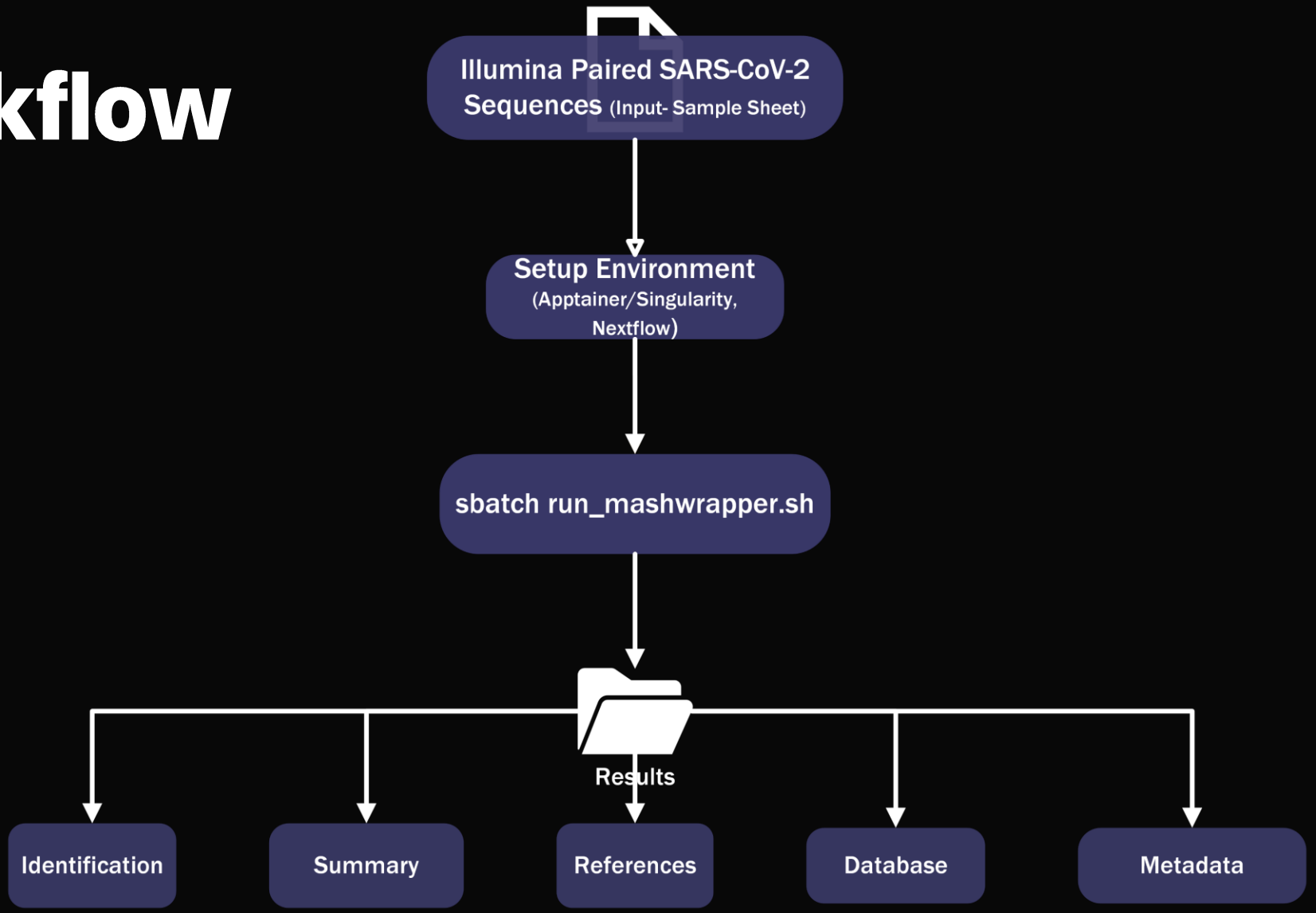
Workflow



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Application

Objective:
Use raw Illumina sequencing reads with mashwrapper to rapidly identify SARS-CoV-2 through k-mer–based comparison against reference genomes.

Application Cont.

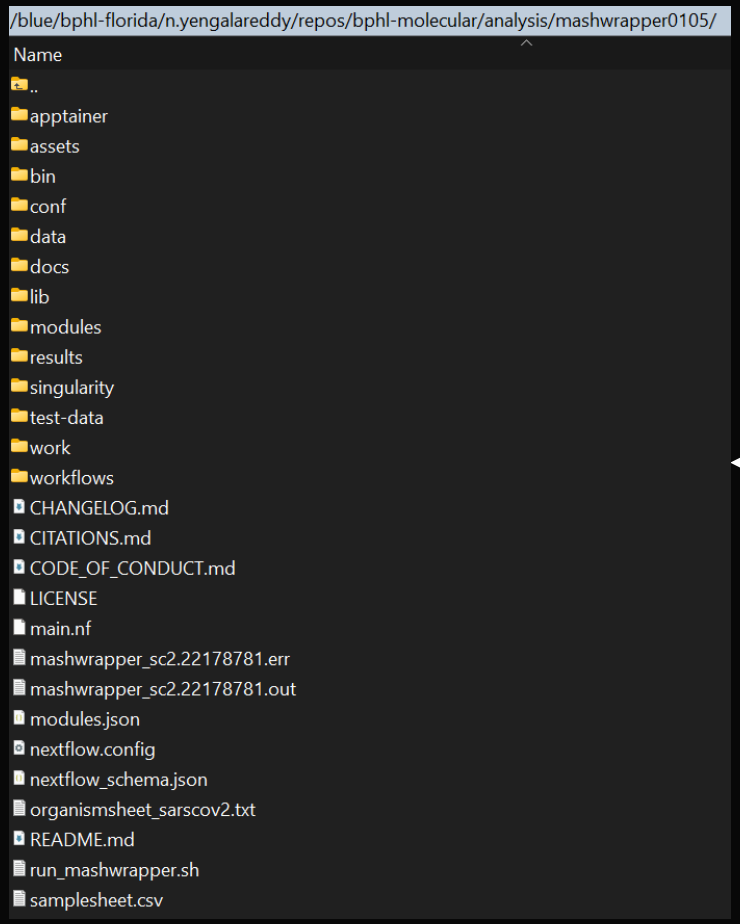
```
cd /blue/bphl-<state>/<user>/repos/bphl-molecular/

git clone https://github.com/CDCgov/mashwrapper

mkdir analysis/

cd analysis/

cp /blue/bphl-<state>/<user>/repos/bphl-molecular/polkapox/
```



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Application Cont.

```
 GNU nano 5.6.1                          run_mashwrapper.sh
#!/usr/bin/bash
#SBATCH --account=bphl-umbrella
#SBATCH --qos=bphl-umbrella
#SBATCH --job-name=mashwrapper_sc2
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=20
#SBATCH --mem=200gb
#SBATCH --time=48:00:00
#SBATCH --output=mashwrapper_sc2.%j.out
#SBATCH --error=mashwrapper_sc2.%j.err
#SBATCH --mail-user=nikhil.yengala@flhealth.gov
#SBATCH --mail-type=FAIL,END

module load nextflow
module load singularity
module load apptainer

#Working Directory
MW="/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105"

#Inputs
SAMPLESHEET="$MW/samplesheet.csv"
ORGSHEET="$MW/organismsheet_sarscov2.txt"

#Output/Work/Cache
OUTDIR="$MW/results"
WORKDIR="$MW/work"
export NXF_SINGULARITY_CACHEDIR="$MW/singularity"
export NXF_APPTAINER_CACHEDIR="$MW/apptainer"

mkdir -p "$OUTDIR" "$WORKDIR" "$NXF_SINGULARITY_CACHEDIR" "$NXF_APPTAINER_CACHEDIR"

#Run
  -nextflow run "$MW" \
  -profile singularity \
  -work-dir "$WORKDIR" \
  --input "$SAMPLESHEET" \
  --get_database "$ORGSHEET" \
  --outdir "$OUTDIR" \
  --custom_config_base "$MW/conf" \
  -resume
```

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/

Name

- ..
- apptainer
- assets
- bin
- conf
- data
- docs
- lib
- modules
- results
- singularity
- test-data
- work
- workflows
- CHANGELOG.md
- CITATIONS.md
- CODE_OF_CONDUCT.md
- LICENSE
- main.nf
- mashwrapper_sc2.22178781.err
- mashwrapper_sc2.22178781.out
- modules.json
- nextflow.config
- nextflow_schema.json
- organismsheet_sarscov2.txt
- README.md
- run_mashwrapper.sh
- samplesheet.csv

```
 GNU nano 5.6.1                organismsheet_sarscov2.txt
Severe acute respiratory syndrome coronavirus 2
```

```
 GNU nano 5.6.1                          samplesheet.csv
sample,fastq_1,fastq_2
J84,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/J84_R1.fastq.gz
J682,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/J682_R1.fastq.
J711,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/J711_R1.fastq.
J886,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/J886_R1.fastq.
M109,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/M109_R1.fastq.
M300,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/M300_R1.fastq.
M301,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/M301_R1.fastq.
M330,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/M330_R1.fastq.
M653,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/M653_R1.fastq.
M908,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/M908_R1.fastq.
T45,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/data/T45_R1.fastq.gz
```

Application Cont.

```
sbatch run_mashwrapper.sh
```

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/

Name
- ..
- apptainer
- assets
- bin
- conf
- data
- docs
- lib
- modules
- results
- singularity
- test-data
- work
- workflows
- CHANGELOG.md
- CITATIONS.md
- CODE_OF_CONDUCT.md
- LICENSE
- main.nf
- mashwrapper_sc2.22178781.err
- mashwrapper_sc2.22178781.out
- modules.json
- nextflow.config
- nextflow_schema.json
- organismsheet_sarscov2.txt
- README.md
- run_mashwrapper.sh
- samplesheet.csv

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/results/combinedOutput/

Name
- ..
- collated_download_genomes.log
- collated_excluded_genomes.txt
- collated_species_id.log
- collated_species_id_results.txt
- database.info

```
SARS-CoV-2 Species ID Tool using Mash
Date and Time = 2025-12-29
Input query file 1: 3711_R1.fastq
Input query file 2: 3711_R2.fastq
Maximum Mash distance (-d): 0.05
Minimum K-mer copy number (-m) to be included in the sketch: 16
K-mer size used for sketching: 25
Mash Database name: myMashDatabase.2025-12-29_17:45:16.msh
mashwrapper version: 3.2.2

Best species match: Severe acute

Top 5 results:
```

Genus	Species	GeneBank Identifier	Mash Dist	% Seq Sim	P-value	Kmer
Severe	acute	GCA_011545545.1	0.00161	99.839	0	29033/31424
Severe	acute	GCA_009858895.3	0.00172	99.828	0	28988/31535
Severe	acute	GCA_011537005.1	0.00172	99.828	0	28988/31535
Severe	acute	GCA_011537985.2	0.00171	99.829	0	28987/31523
Severe	acute	GCA_011537945.2	0.00171	99.829	0	28987/31523

```
SARS-CoV-2 Species ID Tool using Mash
Date and Time = 2025-12-29
Input query file 1: M300_R1.fastq
Input query file 2: M300_R2.fastq
Maximum Mash distance (-d): 0.05
Minimum K-mer copy number (-m) to be included in the sketch: 16
K-mer size used for sketching: 25
Mash Database name: myMashDatabase.2025-12-29_17:45:16.msh
mashwrapper version: 3.2.2

Best species match: Severe acute

Top 5 results:
```

Genus	Species	GeneBank Identifier	Mash Dist	% Seq Sim	P-value	Kmer
Severe	acute	GCA_011545545.1	0.01471	98.529	0	27851/52618
Severe	acute	GCA_009858895.3	0.01485	98.515	0	27777/52758
Severe	acute	GCA_011537005.1	0.01485	98.515	0	27777/52758
Severe	acute	GCA_009938055.1	0.01485	98.515	0	27776/52745
Severe	acute	GCA_011537225.1	0.01482	98.518	0	27776/52697

```
SARS-CoV-2 Species ID Tool using Mash
Date and Time = 2025-12-29
Input query file 1: M330_R1.fastq
Input query file 2: M330_R2.fastq
Maximum Mash distance (-d): 0.05
Minimum K-mer copy number (-m) to be included in the sketch: 24
K-mer size used for sketching: 25
Mash Database name: myMashDatabase.2025-12-29_17:45:16.msh
mashwrapper version: 3.2.2

Best species match: Severe acute
```

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/results/

Name
- ..
- combinedOutput
- downloadedGenomes
- mashDatabase
- mashFiles
- pipeline_info
- resultsByIsolate

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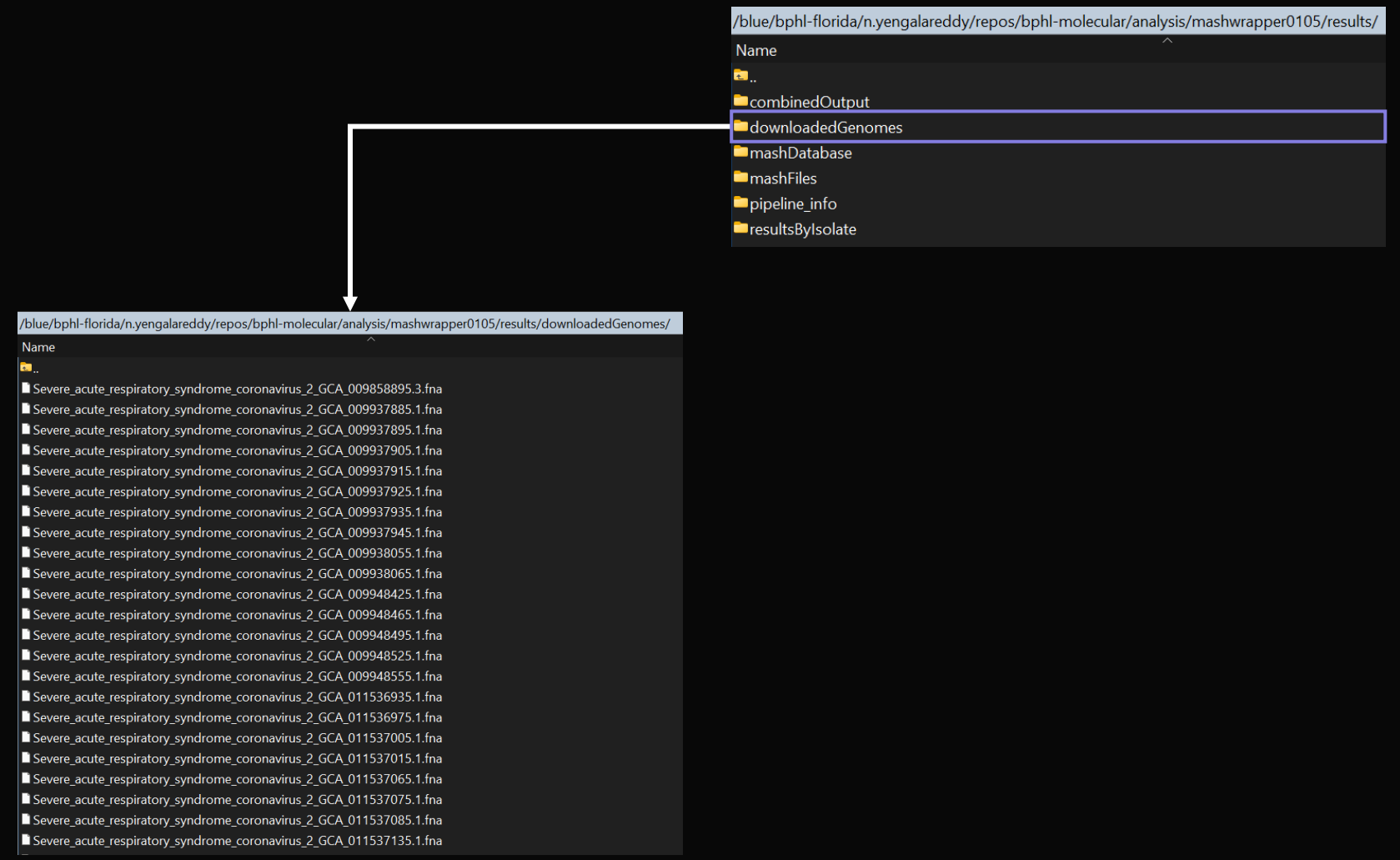
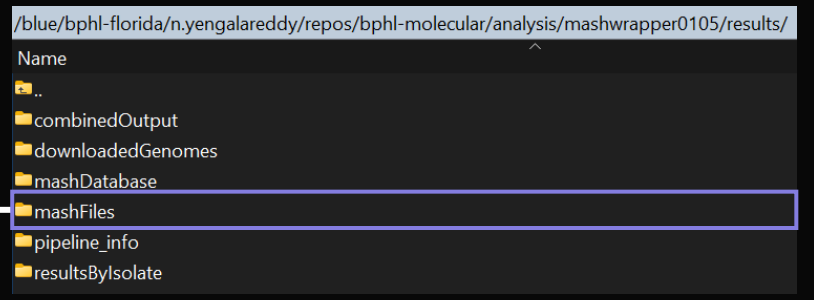
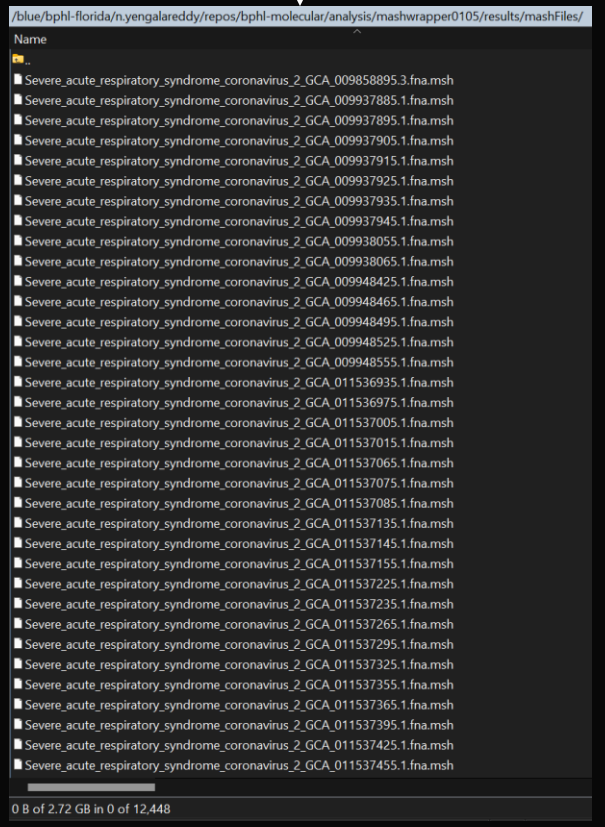
Application Cont.



Application Cont.





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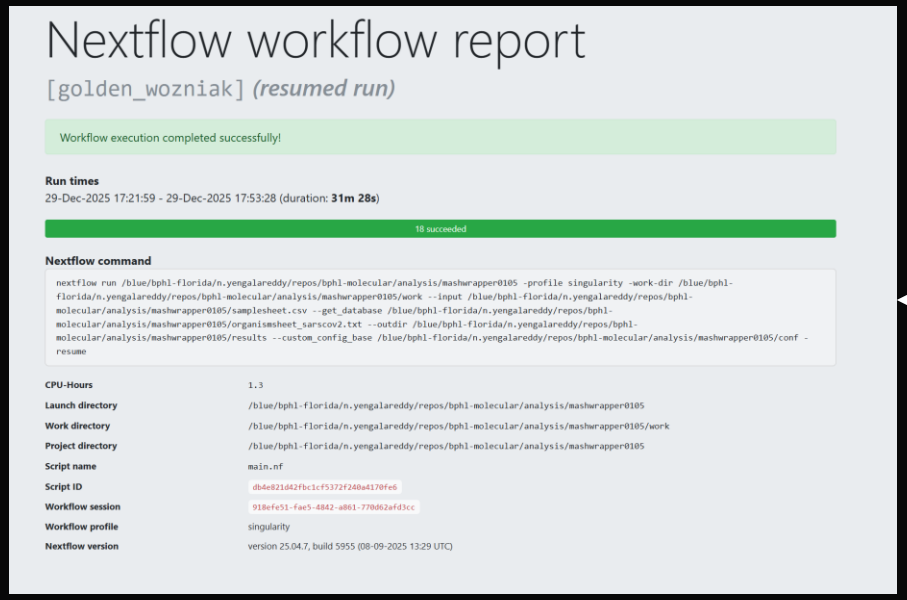
Application Cont.

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/results/

Name
- ..
- combinedOutput
- downloadedGenomes
- mashDatabase
- mashFiles
- pipeline_info
- resultsByIsolate

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/results/pipeline_info/

Name
- ..
- execution_report_2025-12-29_17-21-59.html
- execution_timeline_2025-12-29_17-21-59.html
- execution_trace_2025-12-29_17-21-59.txt
- organismsheet_valid.txt
- pipeline_dag_2025-12-29_17-21-59.svg
- samplesheet.valid.csv
- software_versions.yml
- versions.yml



Nextflow workflow report

[golden_wozniak] *(resumed run)*

Workflow execution completed successfully!

Run times

29-Dec-2025 17:21:59 - 29-Dec-2025 17:53:28 (duration: **31m 28s**)

18 succeeded

Nextflow command

```
nextflow run /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105 -profile singularity -work-dir /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/work --input /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/samplesheet.csv --get_database /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/organismsheet_sarscov2.txt --outdir /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/results --custom_config_base /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/conf -resume
```

CPU-Hours	1.3
Launch directory	/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105
Work directory	/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105/work
Project directory	/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mashwrapper0105
Script name	main.nf
Script ID	db4e821d42fbc1cf5372f240a4170fe6
Workflow session	918efe51-fae5-4842-a861-770d62afd3cc
Workflow profile	singularity
Nextflow version	version 25.04.7, build 5955 (08-09-2025 13:29 UTC)

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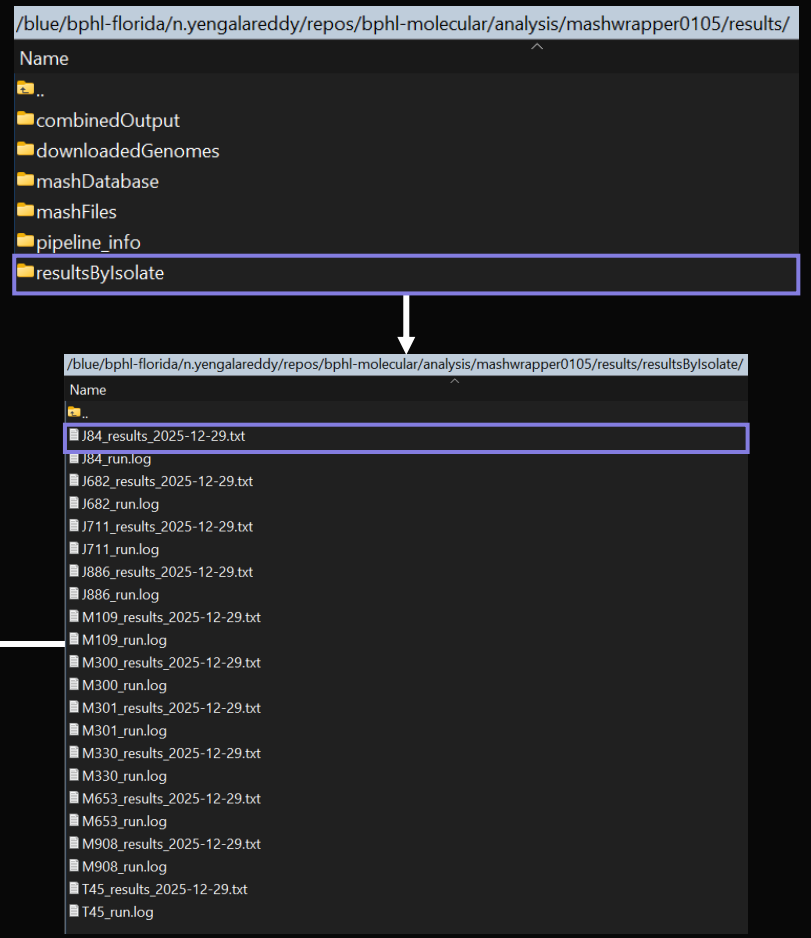
Application Cont.

```
SARS-CoV-2 Species ID Tool using Mash
Date and Time = 2025-12-29
Input query file 1: J84_R1.fastq
Input query file 2: J84_R2.fastq
Maximum Mash distance (-d): 0.05
Minimum K-mer copy number (-m) to be included in the sketch: 13
K-mer size used for sketching: 25
Mash Database name: myMashDatabase.2025-12-29_17:45:16.msh
mashwrapper version: 3.2.2

Best species match: Severe acute

Top 5 results:
```

Genus	Species	GeneBank Identifier	Mash Dist	% Seq Sim	P-value	Kmer
Severe	acute	GCA_011545545.1	0.01266	98.734	0	29703/51828
Severe	acute	GCA_009858895.3	0.01273	98.727	0	29678/51919
Severe	acute	GCA_011537015.1	0.01273	98.727	0	29675/51917
Severe	acute	GCA_011545335.2	0.01273	98.727	0	29675/51909
Severe	acute	GCA_011545285.2	0.01273	98.727	0	29675/51909



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Conclusion

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

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Questions?

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