

29 Sept 2025

# **Neissflow**

**Advanced Molecular Detection  
Southeast Region Bioinformatics**

This resource was made possible through funding provided under the Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases (ELC) Cooperative Agreement (CK24-0002), Project D: Advanced Molecular Detection to the Florida Department of Health. The conclusions, findings, and opinions expressed by authors do not necessarily reflect the official position of the U.S. Department of Health and Human Services, the Public Health Service, or the Centers for Disease Control and Prevention.

# Overview

## Purpose:

- This tool is developed for whole genome sequencing (WGS) analysis of *Neisseria meningitidis* (and subspecies), enabling standardized quality control, genome assembly, species confirmation, molecular typing (MLST), and antimicrobial resistance (AMR) profiling.

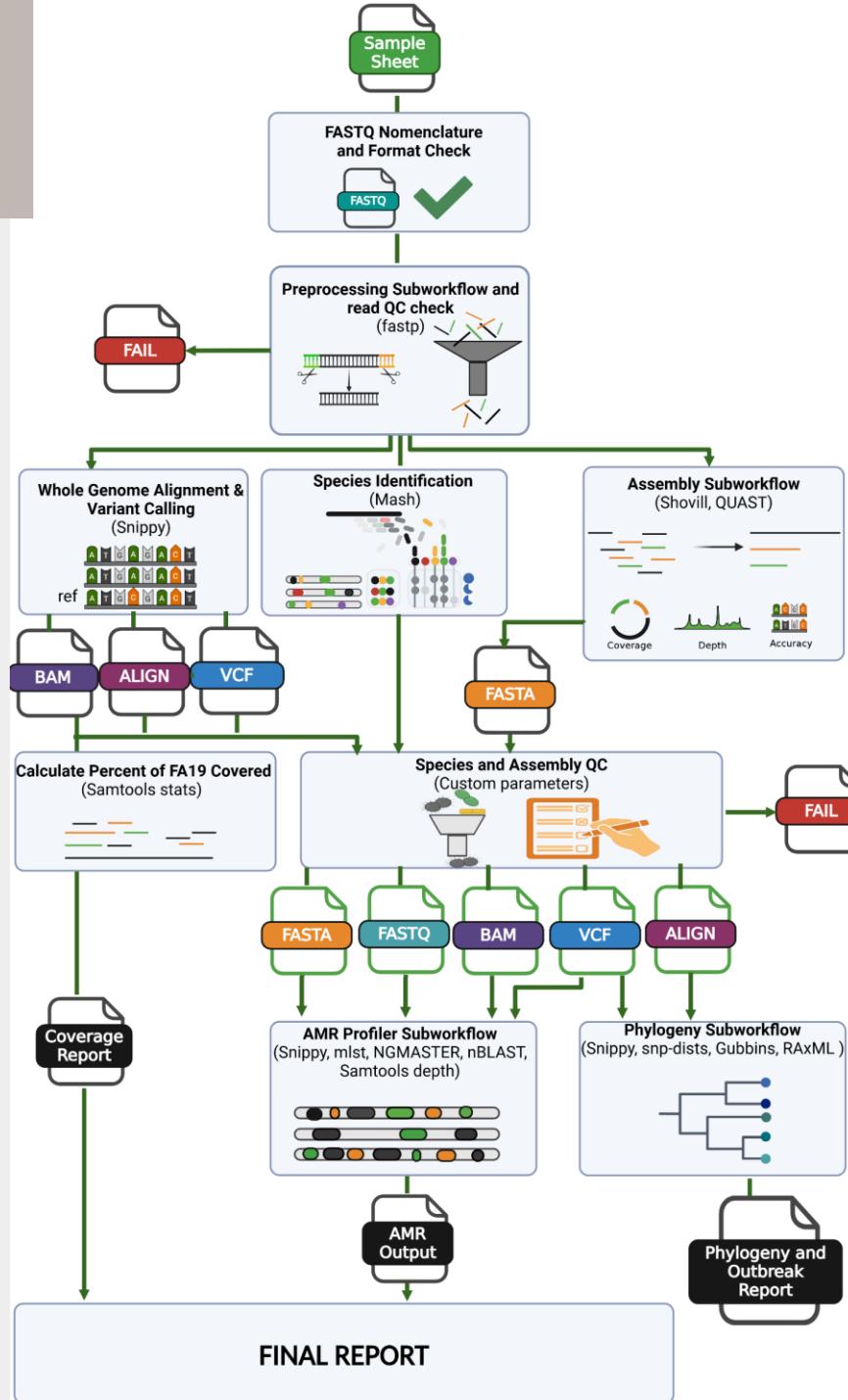
## Usage:

- Can be used by public health laboratories and researchers for molecular typing, outbreak investigation, surveillance, and comparative genomic studies.

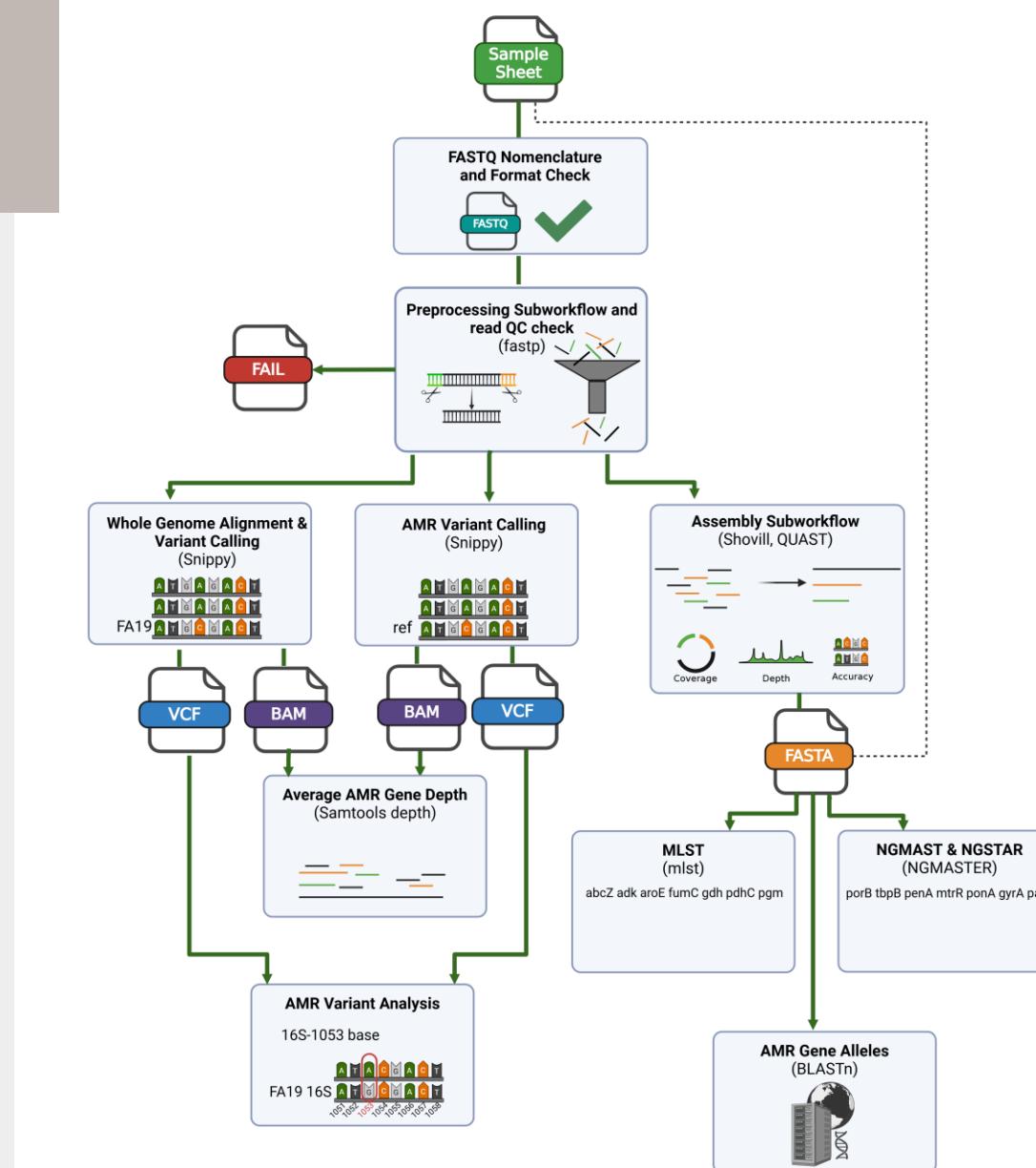
## Dependencies:

- Nextflow
- Singularity/Aptainer
- Java
- Conda
- PubMLST DB

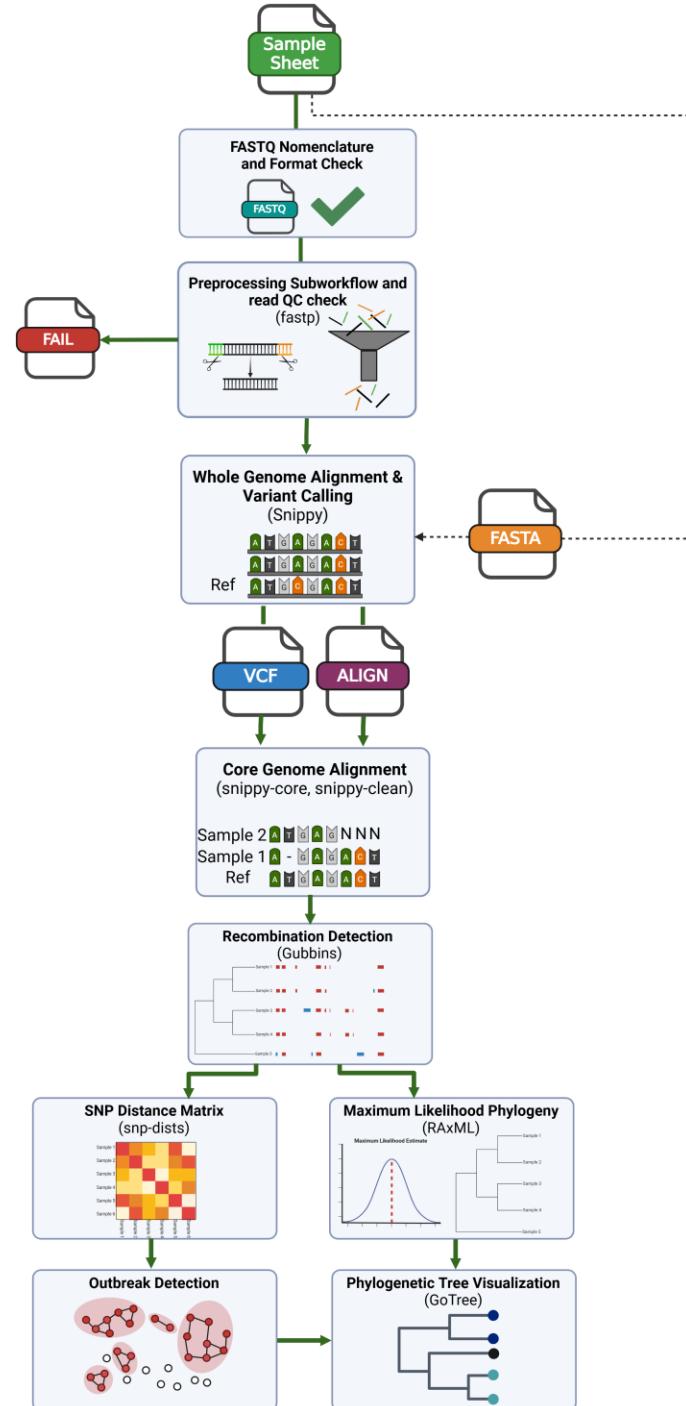
# Workflow



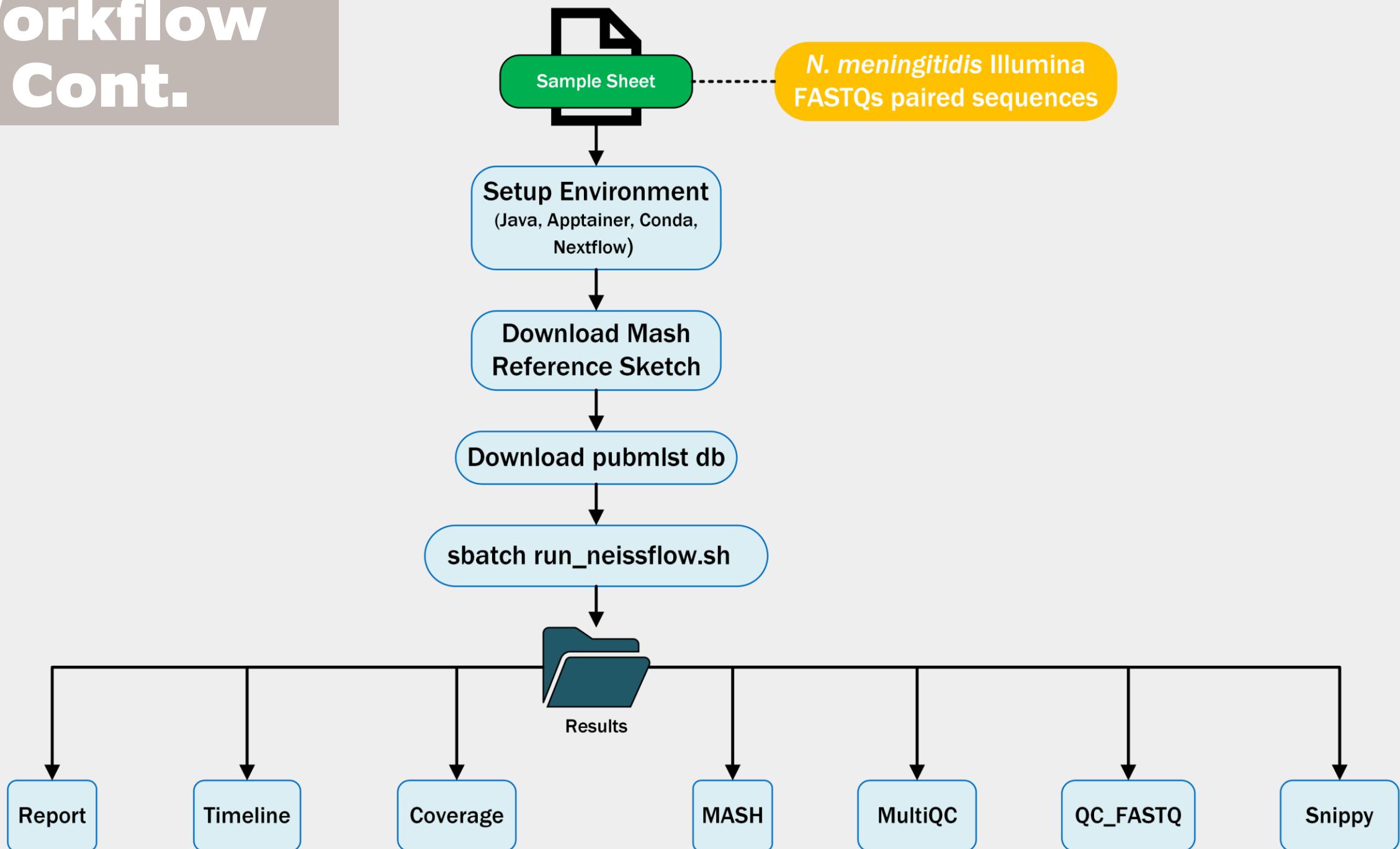
# Workflow Cont.



# Workflow Cont.



# Workflow Cont.



# Application

Acquire *N. meningitidis* Illumina FASTQs paired sequences from NCBI database,  
use Neissflow to analyze the samples

# Application Cont.

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/
Name
..
assets
bin
conf
data
docs
input
logs
modules
pubmlst
results
subworkflows
tmp
work
workflows
CHANGELOG.md
CITATIONS.md
code-of-conduct.md
CONTRIBUTING.md
DISCLAIMER.md
force_slurm_64gb.config
LICENSE
main.nf
modules.json
neissflow.13432233.err
neissflow.13432233.out
nextflow.config
nextflow_schema.json
open_practices.md
README.md
RefSeqSketchesDefaults.msh
rules_of_behavior.md
run_neissflow.sh
thanks.md
tower.yml
```

```
cd blue/bphl-<state>/<user>/repos/bphl-molecular/
git clone https://github.com/CDCgov/
mkdir analysis/
cd analysis/
cp /blue/bphl-<state>/<user>/repos/bphl-molecular/ neissflow/
wget https://gembox.cbcn.umd.edu/mash/RefSeqSketchesDefaults.msh.gz
gunzip RefSeqSketchesDefaults.msh.gz
mlst-download_pub_mlst -d /blue/bphl-<state>/<user>/repos/bphl-molecular/analysis/neissflow/pubmlst
```

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/input/
Name
..
samplesheet.csv
SRR35142010_R1.fastq.gz
SRR35142010_R2.fastq.gz
SRR35142011_R1.fastq.gz
SRR35142011_R2.fastq.gz
SRR35142012_R1.fastq.gz
SRR35142012_R2.fastq.gz
SRR35142013_R1.fastq.gz
SRR35142013_R2.fastq.gz
SRR35142014_R1.fastq.gz
SRR35142014_R2.fastq.gz
SRR35142015_R1.fastq.gz
SRR35142015_R2.fastq.gz
SRR35142016_R1.fastq.gz
SRR35142016_R2.fastq.gz
SRR35142017_R1.fastq.gz
SRR35142017_R2.fastq.gz
SRR35142018_R1.fastq.gz
SRR35142018_R2.fastq.gz
SRR35142019_R1.fastq.gz
SRR35142019_R2.fastq.gz
SRR35142020_R1.fastq.gz
SRR35142020_R2.fastq.gz
```

```
samplesheet.csv
SRR35142010_R1.fastq.gz
SRR35142010_R2.fastq.gz
SRR35142011_R1.fastq.gz
SRR35142011_R2.fastq.gz
SRR35142012_R1.fastq.gz
SRR35142012_R2.fastq.gz
SRR35142013_R1.fastq.gz
SRR35142013_R2.fastq.gz
SRR35142014_R1.fastq.gz
SRR35142014_R2.fastq.gz
SRR35142015_R1.fastq.gz
SRR35142015_R2.fastq.gz
SRR35142016_R1.fastq.gz
SRR35142016_R2.fastq.gz
SRR35142017_R1.fastq.gz
SRR35142017_R2.fastq.gz
SRR35142018_R1.fastq.gz
SRR35142018_R2.fastq.gz
SRR35142019_R1.fastq.gz
SRR35142019_R2.fastq.gz
SRR35142020_R1.fastq.gz
SRR35142020_R2.fastq.gz
```

# Application Cont.

```
#!/bin/bash
#SBATCH --account=bphl-umbrella
#SBATCH --qos=bphl-umbrella
#SBATCH --job-name=neissflow
#SBATCH --cpus-per-task=8
#SBATCH --mem=64gb
#SBATCH --time=48:00:00
#SBATCH --output=neissflow.%j.out
#SBATCH --error=neissflow.%j.err
#SBATCH --mail-user=nikhil.yengala@flhealth.gov
#SBATCH --mail-type=FAIL,END

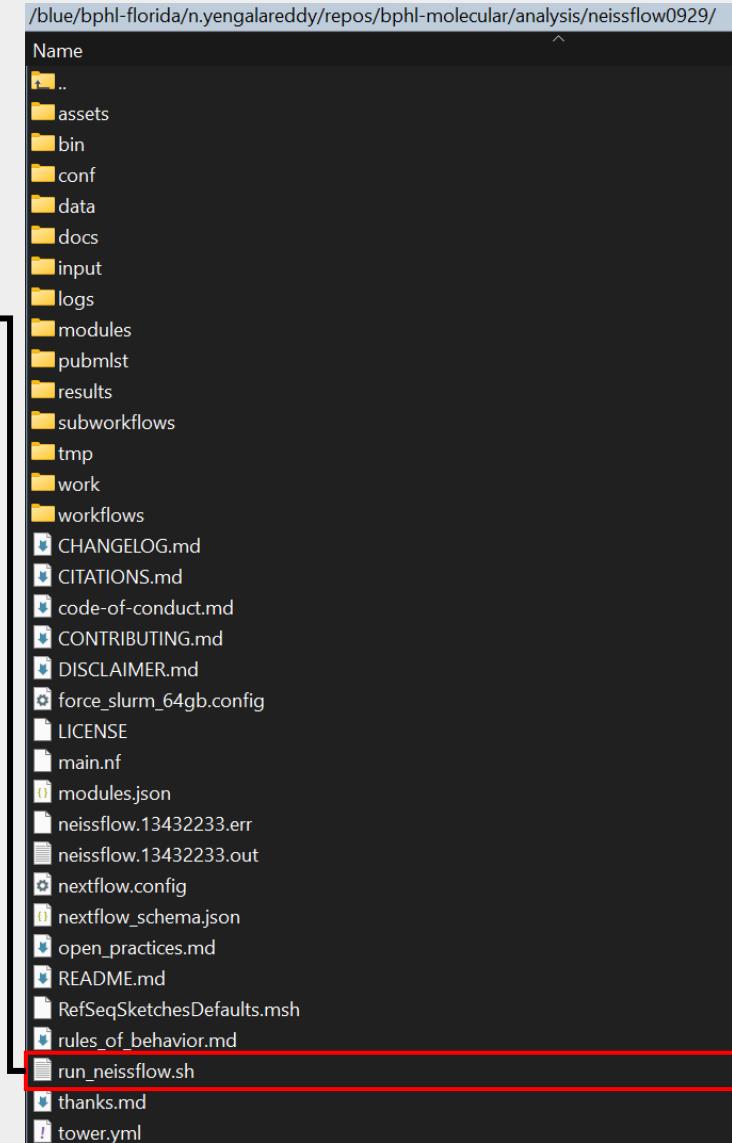
# Modules / env
module load java/20
module load apptainer
module load git
module load conda
module load nextflow/24.04 || true
conda activate Nextflow || true

# Paths
PIPE_DIR="/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929"
OUT_DIR="$PIPE_DIR/results"
INPUT_CSV="$PIPE_DIR/input/samplesheet.csv"
PUBLMLST_DB="$PIPE_DIR/pubmlst"
mash_db="$PIPE_DIR/RefSeqSketchesDefaults.msh" # <-- Mash DB (.msh)
TMPDIR="$PIPE_DIR/tmp"
mkdir -p "$OUT_DIR" "$PIPE_DIR/logs" "$PUBLMLST_DB" "$TMPDIR"
```

```
# Run
cd "$PIPE_DIR"

nextflow run "$PIPE_DIR/main.nf" \
    -profile singularity \
    -c "$PIPE_DIR/force_slurm_64gb.config" \
    --input "$INPUT_CSV" \
    --outdir "$OUT_DIR" \
    --mash_db "$mash_db" \
    --name "ng_run_${date +%Y%m%d_%H%M}" \
    --max_memory '64.GB' \
    --max_cpus 8 \
    --max_time '48.h' \
    -with-report "$OUT_DIR/report.html" \
    -with-trace "$OUT_DIR/trace.txt" \
    -with-timeline "$OUT_DIR/timeline.html"

echo "[INFO] Run finished. Outputs in: $OUT_DIR"
```



# Application Cont.

## Nextflow workflow report

[tiny\_khorana]

Workflow execution completed successfully!

### Run times

19-Sep-2025 19:25:03 - 19-Sep-2025 21:09:41 (duration: **1h 44m 38s**)

71 succeeded

### Nextflow command

```
nextflow run /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/main.nf -profile singularity -c /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/force_slurm_64gb.config --input /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/input/samplesheet.csv --outdir /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/RefSeqSketchesDefaults.msh --name ng_run_20250919_1924 --max_memory 64.GB --max_cpus 8 --max_time 48.h -with-report /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/results/report.html -with-trace /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/results/trace.txt -with-timeline /blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/results/timeline.html
```

### CPU-Hours

1.9

### Launch directory

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929

### Work directory

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/work

### Project directory

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929

### Script name

main.nf

### Script ID

93f13dad4b7f5a348c1bf7f43947ca0d

### Workflow session

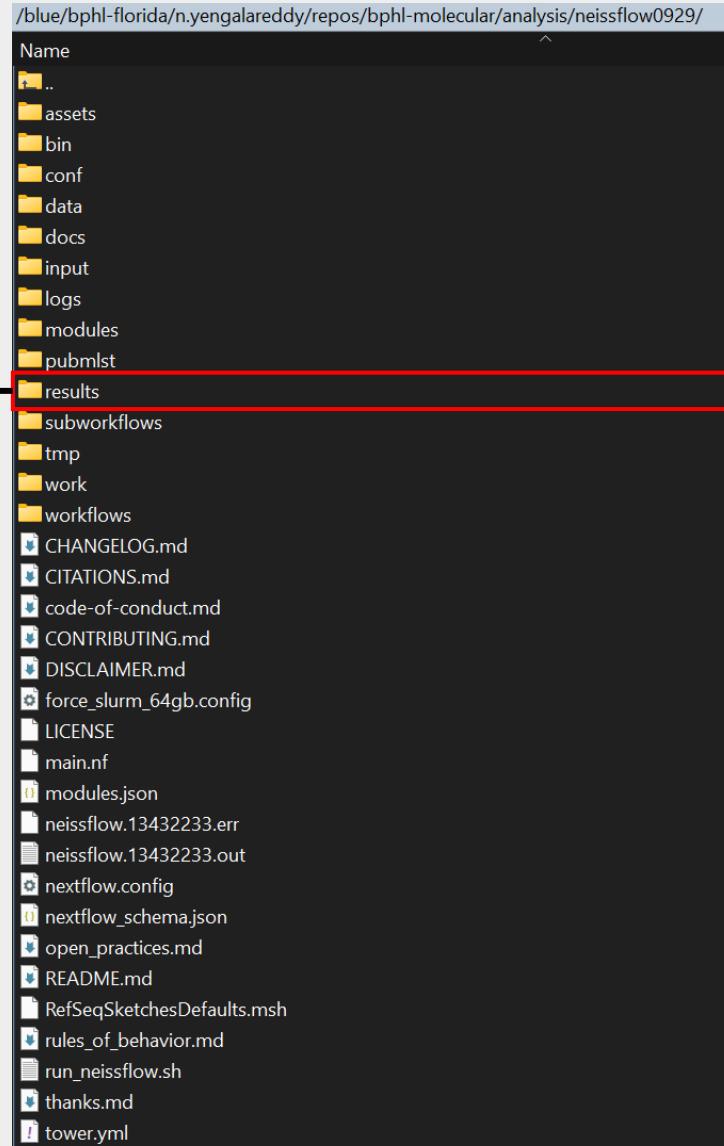
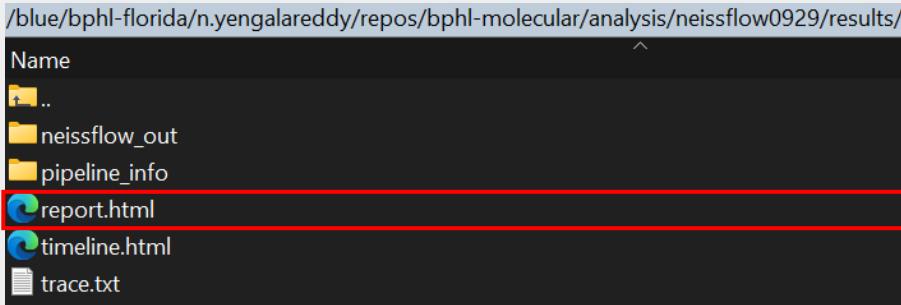
35a9474d-5a84-457d-890f-77d59b943008

### Workflow profile

singularity

### Nextflow version

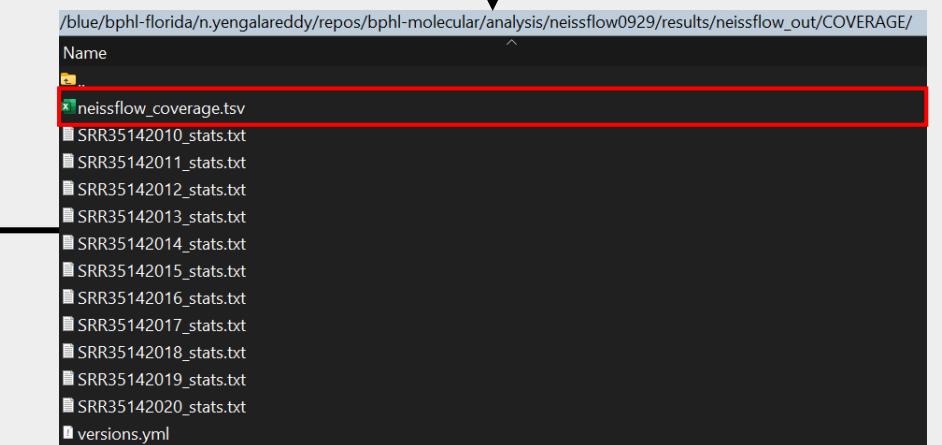
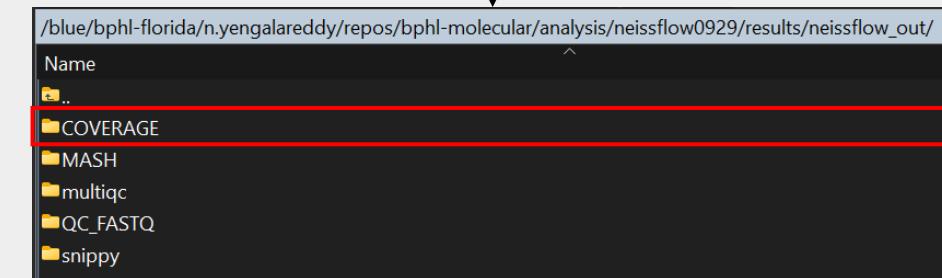
version 24.10.2, build 5932 (27-11-2024 21:23 UTC)



**sbatch run\_neissflow.sh**

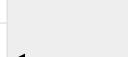
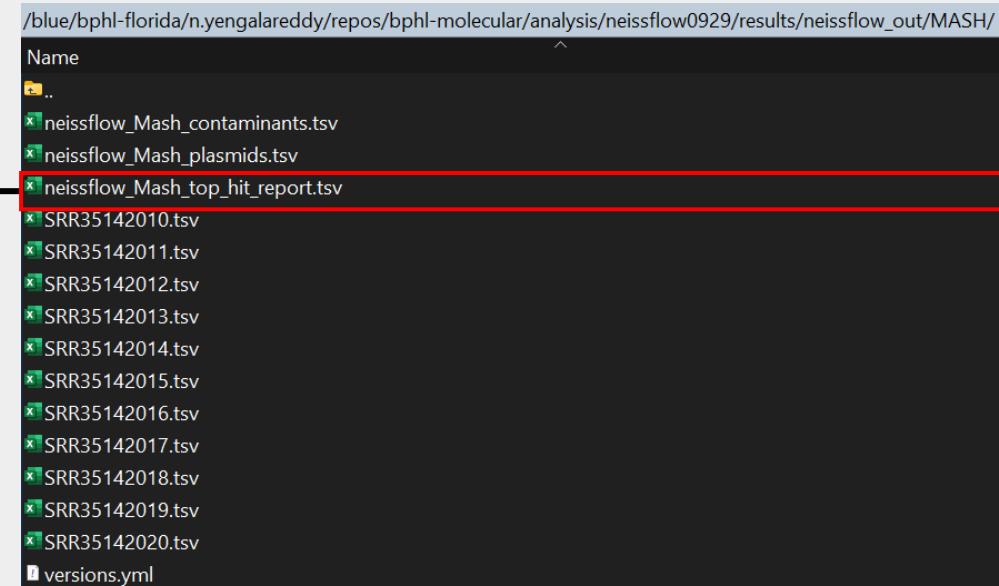
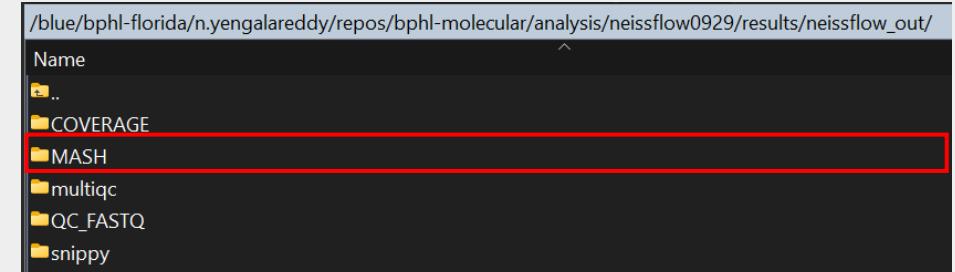
# Application Cont.

ID	%target>10x
SRR35142014	77.89
SRR35142012	78.62
SRR35142015	80.27
SRR35142010	78.07
SRR35142011	76.33
SRR35142016	77.66
SRR35142013	76.24
SRR35142017	77.33
SRR35142019	77.69
SRR35142018	77.88
SRR35142020	77.71



# Application Cont.

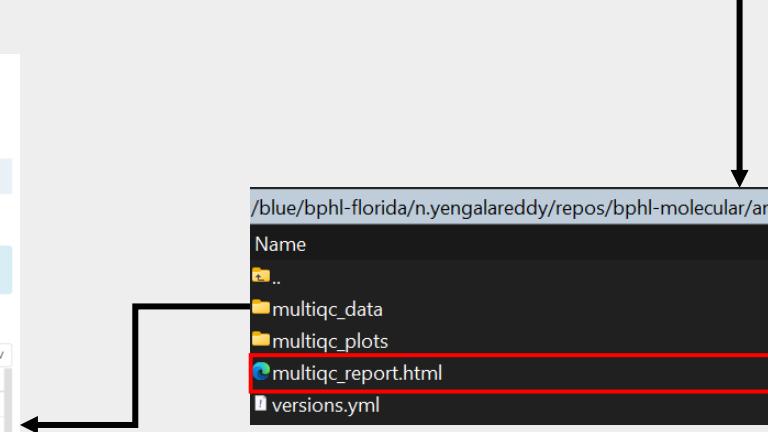
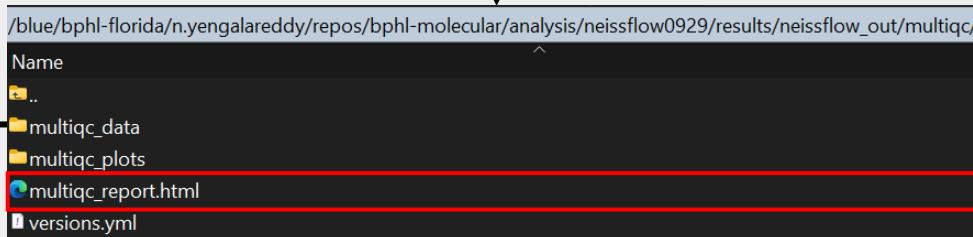
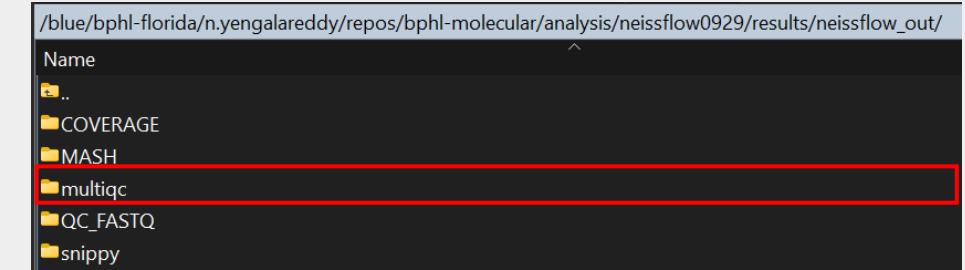
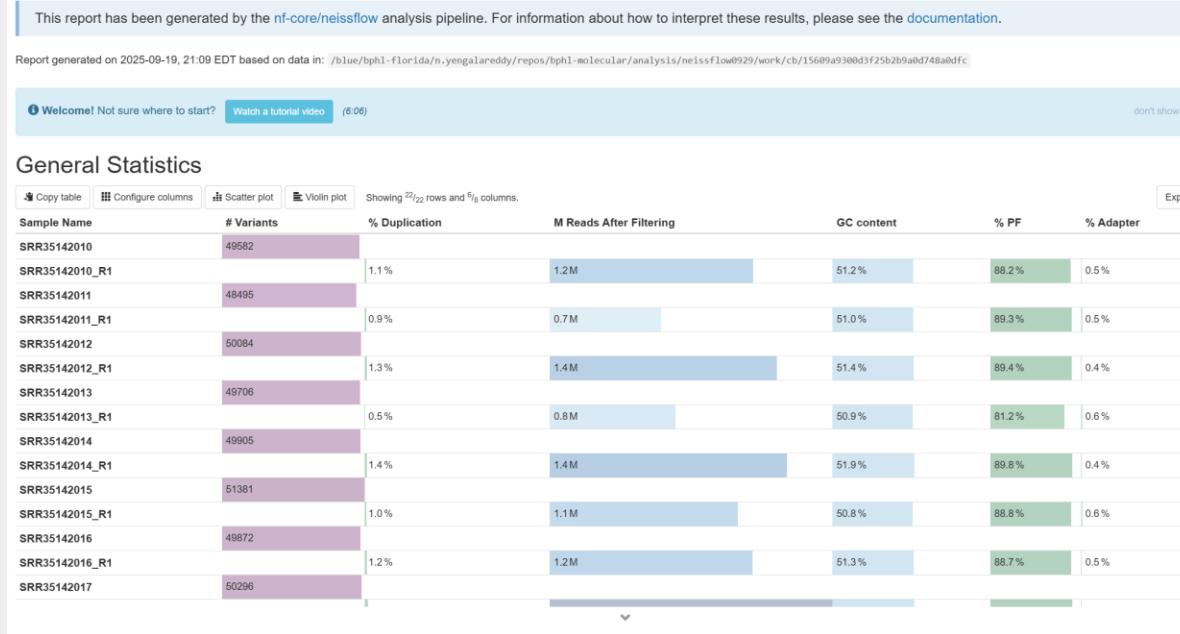
ID	ident	hashes	median_mult	p_val	hit_name
SRR35142011	0.98551	736/1000	37	0	Neisseria_meningitidis_NM2781
SRR35142010	0.988479	784/1000	69	0	Neisseria_meningitidis_M13399
SRR35142013	0.996703	933/1000	79	0	Neisseria_meningitidis_NM2657
SRR35142012	0.989548	802/1000	77	0	Neisseria_meningitidis_M13399
SRR35142015	0.984738	724/1000	60	0	Neisseria_meningitidis_M01_240355
SRR35142014	0.987936	775/1000	83	0	Neisseria_meningitidis_M13399
SRR35142016	0.99756	950/1000	71	0	Neisseria_meningitidis_CU385
SRR35142017	0.993985	881/1000	103	0	Neisseria_meningitidis_NM2795
SRR35142019	0.999905	998/1000	195	0	Neisseria_meningitidis_S0108
SRR35142020	0.99489	898/1000	84	0	Neisseria_meningitidis_NS44
SRR35142018	0.99489	898/1000	87	0	Neisseria_meningitidis_NS44



# Application Cont.



A modular tool to aggregate results from bioinformatics analyses across many samples into a single report.



# Application Cont.



/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/results/neissflow_out/	
Name	^
..	
COVERAGE	
MASH	
multiqc	
QC_FASTQ	
snippy	

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/neissflow0929/results/neissflow\_out/QC\_FASTQ/

Name

..

Reports

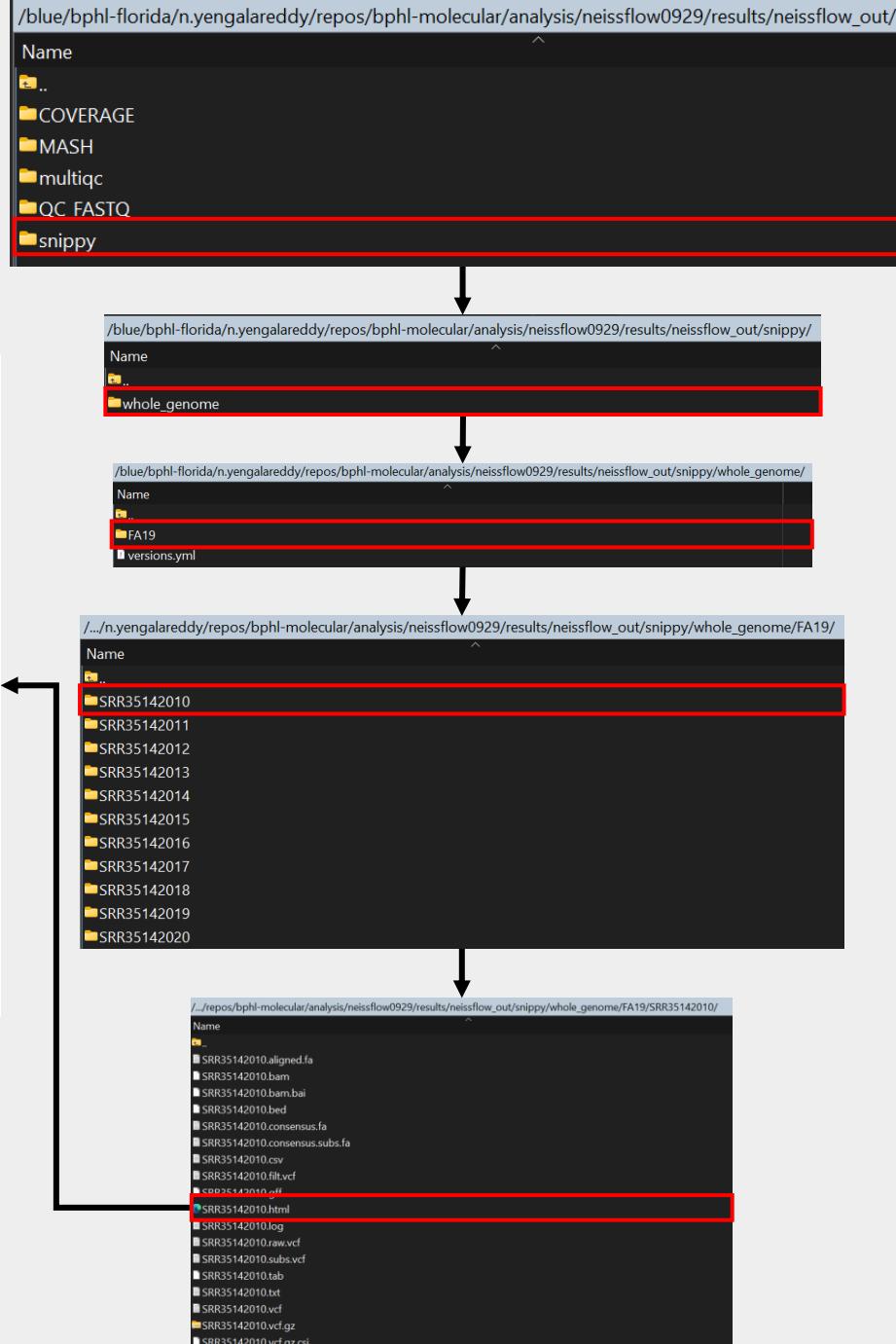
Samples

Name
..
neissflow_failed_qc1.tsv
neissflow_FASTQ_QC_report.tsv
neissflow_passed_qc1.tsv



# Application Cont.

CHROM	POS	TYPE	REF	ALT	EVIDENCE	FTYPE/STRAND	NT_Pos	AA_Pos	EFFECT	LOCUS	TAG	GENE	PRODUCT
CP01202053	10	complex	TCG	T		CD5	+	100_101	missense_variant c.27_28delCCTTT>TAA TAA	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202055	10	complex	TCGG	C		CTCG	-	60_61	missense_variant c.65delTCCTGG>CCCA>p.G25	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202058	132	imp	K	C		C47	0	156_161	missense_variant c.156C>C p.Arg52Arg	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202059	233	imp	C	C		C56	5	207_261	missense_variant c.207C>C p.Ser65Ser	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202060	273	complex	GATCTCA	A		C13	8	248_261	missense_variant c.248A>C p.Gln89Asn	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202061	294	complex	GATCTCA	A		A51	8	281_284	missense_variant c.281A>G p.Gly89Asn	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202064	354	imp	C	A		C41	9	328_361	missense_variant c.328C>A p.Arg107Arg	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202068	356	imp	C	A		GTAAATAAA	GTAAATAAA>ATCAACATC	330_361	missense_variant c.330C>A p.Leu108Leu	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202069	363	complex	ATCAACATC	C		A51	9	337_361	missense_variant c.337_345delCCACACat>CacGTAATATA p.Del17PVal	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202070	395	complex	GTAAATAAA	T		A51	9	351_361	missense_variant c.351A>T p.Qty177Qyl178	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202071	419	complex	CC	T		GTG	7	348_361	missense_variant c.348C>T p.Tyr107Tyr	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202072	446	imp	C	C		C70	9	393_461	missense_variant c.393_394delT p.Ile133Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202074	514	imp	C	A		C56	5	420_461	missense_variant c.420C>G p.Arg140Arg	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202075	549	imp	ATCAGAAGGTTA	A		ATCAGAAGGTTA	ATCAGAAGGTTA>ACAAAAAGCCT 36 OCAGGAAGGTTA>A	129_461	missense_variant c.129A>G p.Phe109Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202076	573	imp	AAA	TCG		TGG	11	100_129	missense_variant c.100A>G p.Lys34Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202088	488	imp	C	C		C47	5	649_861	missense_variant c.649_650delAAATCG>p.Lys21Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202089	495	imp	C	C		C31	5	660_861	missense_variant c.660C>G p.Arg220Arg	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202090	496	imp	C	C		C56	5	669_861	missense_variant c.669C>G p.Ile223Ala	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202093	448	imp	C	A		A51	9	672_861	missense_variant c.672C>A p.Thr227Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202095	495	imp	K	A		A51	9	678_861	missense_variant c.678C>A p.Thr227Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202097	493	imp	K	A		A58	9	680_861	missense_variant c.680C>A p.Thr227Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202098	511	imp	C	A		A54	9	681_861	missense_variant c.681C>A p.Thr227Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202099	569	imp	C	T		T10	9	724_861	missense_variant c.724C>T p.Thr227Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202099	1143	imp	C	A		A132	9	726_861	missense_variant c.726C>A p.Thr227Ter	V700_00001	BpA_1	Lactoferrin binding protein A precursor	
CP01202099	1689	imp	G	A		A38	9	820_827	missense_variant c.820C>T p.Thr227Ter	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1700	imp	G	A		A38	9	858_827	missense_variant c.858C>T p.Thr227Ter	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1712	complex	GGG	CA		CA41	0	971_827	missense_variant c.971_976delAAATCG>p.Lys13Ala	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1412	imp	G	T		T49	5	367_827	missense_variant c.367C>G p.Gly196Gly	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1428	complex	AGAC	CGAT		CGAT	48	368_827	missense_variant c.368C>G p.Ile197Val	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1521	imp	G	T		T49	5	373_827	missense_variant c.373C>G p.Gly196Gly	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1530	imp	C	T		T49	5	448_827	missense_variant c.448C>G p.Lys197Lys	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1550	imp	A	G		G42	5	129_827	missense_variant c.129A>G p.Thr227Ter	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1700	complex	GTGAA	ATGAA		ATGAA	0	393_827	missense_variant c.393_394delAAATCG>p.Lys13Ter	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	1841	imp	C	A		A49	5	728_827	missense_variant c.728C>A p.Thr227Ter	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2163	imp	C	A		C70	5	361_827	missense_variant c.361C>A p.Thr227Ter	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2168	imp	C	GTC		GTC	0	349_827	missense_variant c.349C>G p.Thr227Ter	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2170	imp	C	G		G62	0	306_827	missense_variant c.306C>G p.Asp193Asn	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2171	imp	C	A		A58	0	289_827	missense_variant c.289C>A p.Asp193Asn	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2178	imp	CA	T		T57	0	282_827	missense_variant c.282C>T p.Asp193Asn	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2180	imp	C	A		T57	0	291_827	missense_variant c.291C>A p.Asp193Asn	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2181	imp	C	A		T57	0	271_827	missense_variant c.271C>A p.Asp193Asn	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2183	imp	CG	CA		CA19	0	271_827	missense_variant c.271C>A p.Asp193Asn	V700_00002	NgC	phosphoglycerate kinase (luminal isoform)	
CP01202099	2203	imp	CG	CA		CA19TG	0	444_492	missense_variant c.444C>G p.Gly148Gly	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2148	imp	D	A		A72	0	393_492	missense_variant c.393C>T p.Cys131Cys	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2157	imp	D	A		A72	0	393_492	missense_variant c.393C>T p.Cys131Cys	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2200	imp	E	A		Q71	0	382_492	missense_variant c.382C>A p.Leu238Leu	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2272	imp	D	A		F72	0	334_492	missense_variant c.334C>A p.Val112Ile	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2311	imp	D	A		C74	0	247_492	missense_variant c.247C>A p.Thr117Ter	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2329	imp	Z	C		C41	9	223_492	missense_variant c.223C>A p.Thr117Ter	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2481	imp	A	C		O71	0	184_492	missense_variant c.184C>G p.Thr117Ter	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2544	imp	D	A		T76	0	184_492	missense_variant c.184C>G p.Thr117Ter	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2599	imp	AA	GG		GGD	11	184_492	missense_variant c.184C>G p.Thr117Ter	V700_00003	GrB	Transcription elongation factor GrB	
CP01202099	2708	imp	G	C		C46	0	147_492	missense_variant c.147C>G p.Ile95Gly	V700_00004	GrB	Amidophosphoenol transferase	
CP01202099	2708	imp	C	T		T46	0	143_492	missense_variant c.143C>T p.Arg192Ter	V700_00004	GrB	Amidophosphoenol transferase	
CP01202099	2709	imp	C	T		T57	0	134_492	missense_variant c.134C>T p.Leu144Leu	V700_00004	GrB	Amidophosphoenol transferase	



# Conclusion

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



# Advanced Molecular Detection

## Southeast Region Bioinformatics

# Questions?

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