### PHoeNIX

Advanced Molecular Detection
Southeast Region Bioinformatics

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#### Updates

- Reminder don't forget to register for the BTL AMD virtual training
  - Open to all Southeast regional epis, laboratorians, and bioinformaticians
  - No limit to registrants!
  - Fridays for 15 weeks starting July 11



#### What is PHoeNIx?

#### **Overview**

• CDC developed Nextflow pipeline for analyzing bacterial genomes with a focus on AMR.

#### <u>Usage</u>

• Used by public health labs and researchers to analyze bacterial genomes, understand outbreaks, track pathogen evolution, and support studies on antimicrobial resistance and treatment response.

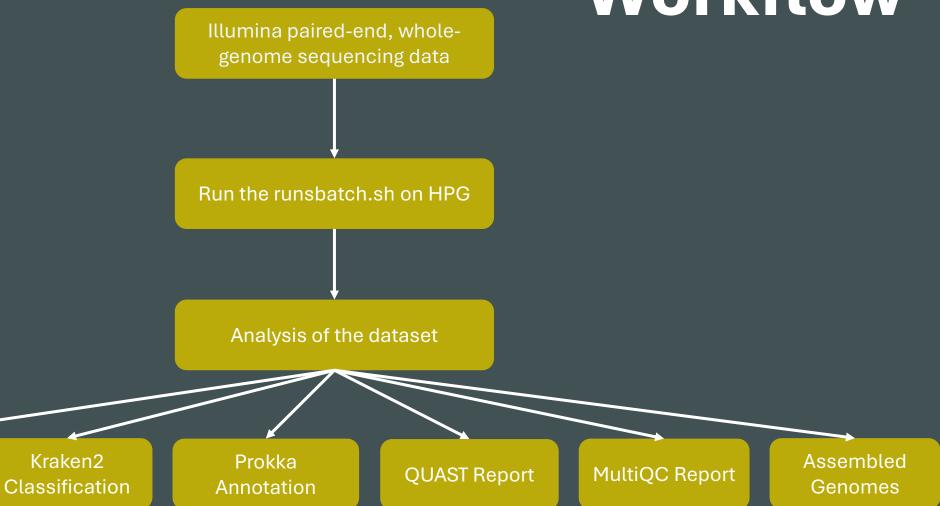
#### **Dependencies**

- FASTQC
- MultiQC
- Kraken2
- Nextflow
- Singularity



FastQC Report

#### Workflow



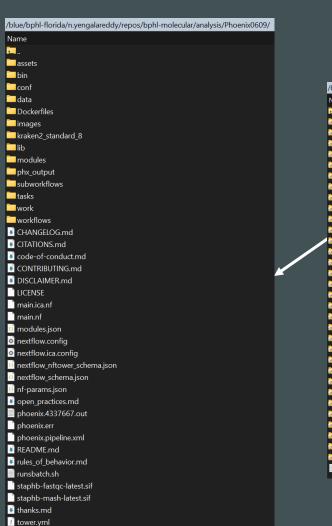


### Application

#### **Objective**

Use an unknown dataset and analyze the dataset using PHoeNIx pipeline





```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Phoenix0609/data/
Name
J4 1.fastq.qz
J4 2.fastq.qz
3 1.fastq.qz
37_2.fastq.gz
38_1.fastq.gz
J8 2.fastq.qz
🧰 J9_1.fastq.gz
39_2.fastq.gz
3 J17 1.fastq.qz
J17_2.fastq.gz
36_1.fastq.gz
36_2.fastq.gz
J40_1.fastq.gz
= J40 2.fastq.gz
352 1.fastq.gz
J52_2.fastq.gz
≔ J100_1.fastq.gz
J100 2.fastq.qz
= J102 2.fastq.gz
J120_2.fastq.gz
J217_2.fastq.gz
3414 2.fastq.qz
J756_1.fastq.gz
= J756_2.fastq.gz
3762 1.fastq.qz
🧺 J762_2.fastq.gz
= J780 1.fastq.qz
3780 2.fastq.gz
samplesheet.csv
```

```
cd /blue/bphl-<state>/<user>/repos/bphl-
molecular/
git clone https://github.com/CDCgov/phoenix
mkdir analysis/
cd analysis/
cp /blue/bphl-<state>/<user>/repos/bphl-
molecular/Phoenix/*
Mkdir data/
cp /path/to/data/*.fastq.gz data/
```



J780\_1.fastq.gz J780\_2.fastq.gz samplesheet.csv





#### **Application Cont.**

cd /blue/bphl-<state>/<user>/repos/bphl-molecular/Phoenix0609/\*

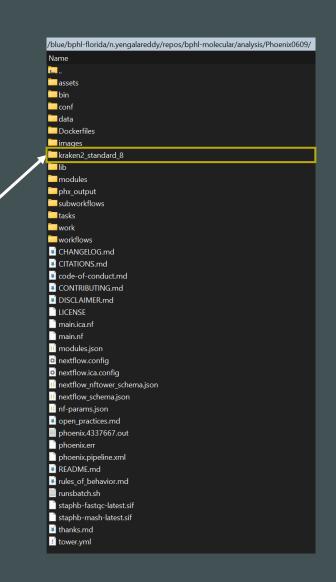
mkdir kraken2\_standard\_8/

wget https://genomeidx.s3.amazonaws.com/kraken/k2\_standard\_ 08gb\_20250402.tar.gz

tar -xvzf k2\_standard\_08gb\_20250402.tar.gz

Delete k2\_standard\_08gb\_20250402.tar.gz after

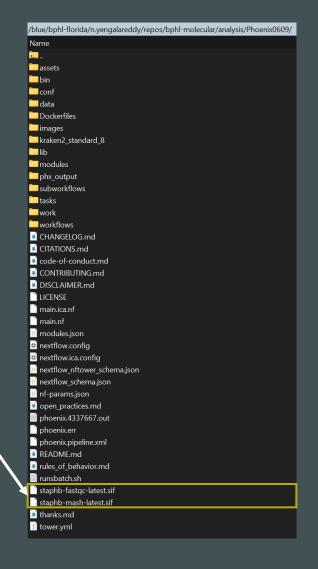
Check for hash.k2d, opts.k2d, and taxo.k2d files





#### **Application Cont.**

singularity pull staphb-mash-latest.sif
docker://staphb/mash:latest





```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Phoenix0609/
Name
conf
data
Dockerfiles
images
kraken2 standard 8
ilib 🗀
modules
phx output
subworkflows
tasks
work
workflows
CHANGELOG.md
CITATIONS.md
code-of-conduct.md
CONTRIBUTING.md
■ DISCLAIMER.md
LICENSE
main.ica.nf
main.nf
modules.ison
nextflow.config
nextflow.ica.config
nextflow nftower_schema.json
nextflow_schema.json
of-params.json
open_practices.md
phoenix.4337667.out
phoenix.err
phoenix.pipeline.xml
README.md
rules_of_behavior.md
runsbatch.sh
staphb-fastqc-latest.sif
staphb-mash-latest.sif
thanks.md
! tower.yml
```

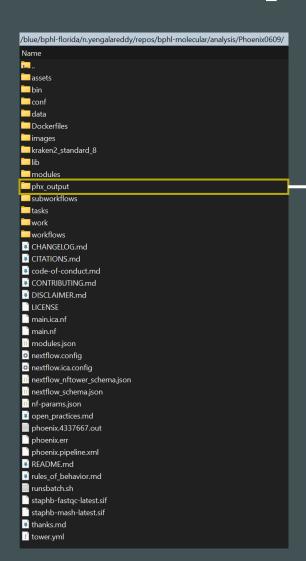
```
// Max resource options
   // Defaults only, expecting to be overwritten
   max memory
                              = '128.GB'
                              = 16
   max_cpus
   max_time
                              = '240.h'
process {
// Add this block to customize Kraken2 resource usage
 withName: KRAKEN2_TRIMD {
    memory = check max(128.GB, 'memory')
    maxForks = 1
        args = '--use-names'
 publishDir = [
             path: { "${params.outdir}/${meta.id}/kraken2_trimd" },
             pattern: '*{classifiedreads.txt,.summary.txt}'
  withName: MASH DIST {
    container = "${baseDir}/staphb-mash-latest.sif"
 withName: FASTQCTRIMD {
   container = "${baseDir}/staphb-fastqc-latest.sif"
 shell = ['/bin/bash', '-euo', 'pipefail']
// Load base.config by default for all pipelines
includeConfig 'conf/base.config'
includeConfig 'conf/modules.config'
// Load nf-core custom profiles from different Institutions
  includeConfig "${params.custom_config_base}/nfcore_custom.config"
catch (Exception e) {
   System.err.println("WARNING: Could not load nf-core/config profiles: ${params.custom_config_base}/nfcore_custom.config")
```

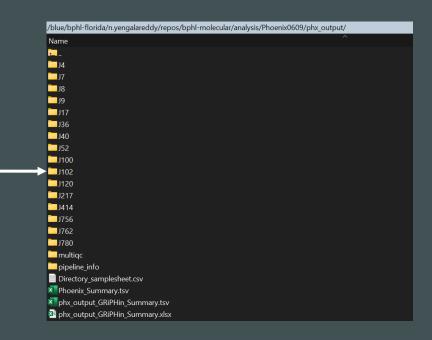


```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Phoenix0609/
Name
bin
conf
data
Dockerfiles
images
kraken2 standard 8
lib
modules
phx output
subworkflows
tasks
work
workflows
CHANGELOG.md
CITATIONS.md
code-of-conduct.md
CONTRIBUTING.md
■ DISCLAIMER.md
LICENSE
main.ica.nf
main.nf
modules.ison
nextflow.config
nextflow.ica.config
nextflow nftower schema.json
nextflow schema.json
of-params.json
open_practices.md
phoenix.4337667.out
phoenix.err
phoenix.pipeline.xml
■ README.md
rules of behavior.md
runsbatch.sh
staphb-fastqc-latest.sif
staphb-mash-latest.sif
thanks.md
! tower.yml
```

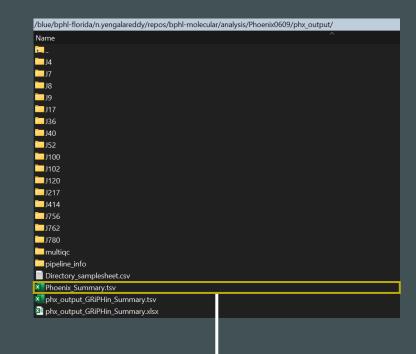
```
#!/bin/bash
 #SBATCH --account=bphl-umbrella
 #SBATCH --gos=bphl-umbrella
 #SBATCH --job-name=Phoenix
 #SBATCH --ntasks=1
 #SBATCH --cpus-per-task=10
 #SBATCH --mem=256gb
 #SBATCH --time=48:00:00
 #SBATCH --output=phoenix.%j.out
 #SBATCH --error=phoenix.err
 #SBATCH --mail-user=nikhil.yengala@flhealth.gov
 #SBATCH --mail-type=FAIL,END
module load singularity
 export SINGULARITY_BINDPATH="/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Phoenix0609/work"
 export NXF SINGULARITY CACHEDIR="/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Phoenix0609/work/singularity"
 SAMPLESHEET="/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Phoenix0609/data/samplesheet.csv"
 KRAKEN_DB="/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Phoenix0609/kraken2_standard_8/"
 nextflow run main.nf -profile singularity \
    -entry PHOENIX \
   --input "$SAMPLESHEET" \
   --kraken2db "$KRAKEN DB" \
```





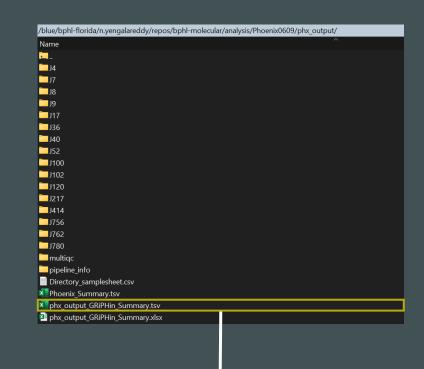






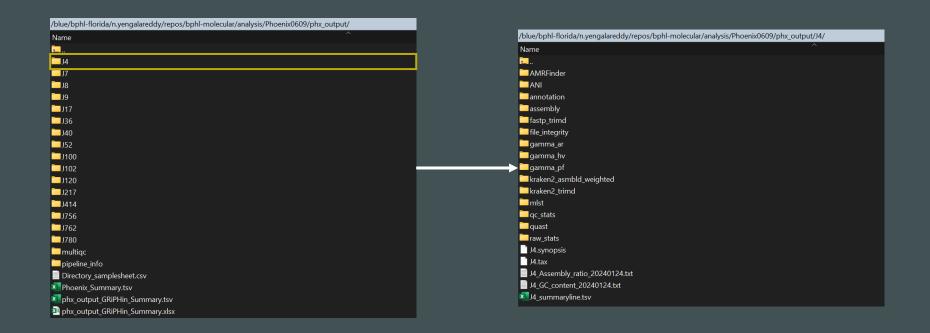
		me Warning_Count E	stimated_Coverage Ge	enome_Length Assembly_Ratio_(STDe	ev) #_of_Scaffolds_>500bp GC_% Species	Taxa_Confidence Taxa	a_Coverage Taxa_Source Kraken2_Trimd	Kraken2_Weighte	d MLST_Scheme_1	MLST_1	MLST_Scheme_2	MLST_2	GAMMA_Beta_Lactam_Resistance_Genes
J4 P.	ASS	1	130.2	5263379 1.0308 (.4432)	179 50.8 Escherichia coli	99.89 ANI_match	96.96 ANI_REFSEQ Escherichia(14.22%) coli(13.08%)	Unknown	ecoli(Achtman)	ST10	ecoli_2(Pasteur)	Novel_allel	le AmpC1_Ecoli_FN649414,Penicillin_Binding_Protein_Ecoli_CP002291,ampH_Ecoli_AP012030,blaEC_U00096.
J7 P.	ASS	2	80.52	5328404 1.0435 (.6263)	246 50.5 Escherichia coli	99.95 ANI_match	98.98 ANI_REFSEQ Escherichia(16.87%) coli(13.30%)	Unknown	ecoli(Achtman)	ST21	ecoli_2(Pasteur)	ST481	AmpC1_Ecoli_FN649414,Penicillin_Binding_Protein_Ecoli_CP002291,ampH_Ecoli_AP012030,blaEC-18_NG_0
J8 P	ASS	2	100.4	5505983 1.0783 (1.1266)	294 50.3 Escherichia coli	99.66 ANI_match	96.31 ANI_REFSEQ Escherichia(17.96%) coli(12.65%)	Unknown	ecoli(Achtman)	ST29	ecoli_2(Pasteur)	ST481	AmpC1_Ecoli_FN649414,Penicillin_Binding_Protein_Ecoli_CP002291,ampH_Ecoli_AP012030,blaEC-18_NG_0
J9 P.	ASS	1	109.1	4684746 0.9175 (1.1868)	57 50.7 Escherichia coli	99.46 ANI_match	96.09 ANI_REFSEQ Escherichia(7.71%) coli(6.54%)	Unknown	ecoli(Achtman)	ST1248	ecoli_2(Pasteur)	Novel_allel	le AmpC1_Ecoli_FN649414,Penicillin_Binding_Protein_Ecoli_CP002291,ampH_Ecoli_AP012030,blaEC-18_NG_0
J17 P		2	89.77	5350037 1.0478 (.6873)	267 50.5 Escherichia coli	99.86 ANI_match	99.16 ANI_REFSEQ Escherichia(15.96%) coli(11.37%)	Unknown	ecoli(Achtman)	ST17	ecoli_2(Pasteur)	Novel_allel	le AmpC1_Ecoli_FN649414,Penicillin_Binding_Protein_Ecoli_CP002291,ampH_Ecoli_AP012030,blaEC-18_NG_0
J36 P	ASS	0	86.57	5427905 0.9678 (.6836)	51 57.5 Klebsiella pneumoniae	99.93 ANI_match	95.57 ANI_REFSEQ Klebsiella(85.17%) pneumoniae(3.08%)	Unknown	kpneumoniae	ST11	-	-	ampH_CP003785,blaCTX-M-27_NG_048976.1,blaKPC-3_NG_049257.1,blaSHV-11_HM751098,blaTEM-1A_1_
J40 P	ASS	0	60.2	4872278 0.9778 (.4291)	70 55.1 Enterobacter hormaeche	i 99.83 ANI_match	96.17 ANI_REFSEQ Enterobacter(84.33%) hormaechei(19.17%)	Unknown	ecloacae	ST102	-	-	blaACT-47_NG_050710.1,blaKPC-3_NG_049257.1,blaSHV-7_NG_050104.1
J52 P	ASS	2	119.7	5051000 0.9892 (.1550)	137 50.5 Escherichia coli	99.60 ANI_match	89.31 ANI_REFSEQ Escherichia(10.20%) coli(8.59%)	Unknown	ecoli(Achtman)	ST10	ecoli_2(Pasteur)	ST367	AmpC1_Ecoli_FN649414,Penicillin_Binding_Protein_Ecoli_CP002291,ampH_Ecoli_AP012030,blaEC_U00096.
J100 P	ASS	1	87.21	4882908 0.9563 (.6285)	99 50.6 Escherichia coli	99.98 ANI_match	97.98 ANI_REFSEQ Escherichia(11.17%) coli(9.41%)	Unknown	ecoli(Achtman)	ST410	ecoli_2(Pasteur)	Novel_allel	le AmpC1_Ecoli_FN649414,Penicillin_Binding_Protein_Ecoli_CP002291,ampH_Ecoli_AP012030,blaCMY-2_NG_0
J102 P	ASS	0	69.61	5752511 1.0257 (.5467)	67 56.8 Klebsiella pneumoniae	99.95 ANI_match	93.26 ANI_REFSEQ Klebsiella(80.79%) pneumoniae(13.06%)	Unknown	kpneumoniae	ST307	-	-	ampH_CP003785,blaCTX-M-15_NG_048935.1,blaKPC-2_NG_049253.1,blaOXA-1_NG_049392.1_blaOXA-1-lik
J120 P	ASS	1	54.22	7316659 1.1005 (1.5743)	128 65.4 Pseudomonas aeruginosa	99.93 ANI_match	92.23 ANI_REFSEQ Pseudomonas(93.43%) aeruginosa(21.96%)	Unknown	paeruginosa	ST244		-	blaOXA-675_NG_062270.1_blaOXA-1-like,blaOXA-847_NG_066520.1_blaOXA-50-like,blaPAO_1_AY083595,blaPAO_1
J217 P	ASS	0	93.49	3873477 0.9715 (.4391)	65 39 Acinetobacter baumannii	99.87 ANI_match	96.66 ANI_REFSEQ Acinetobacter(95.11%) baumannii(4.81%)	Unknown	abaumannii(Oxford	d) STNovel_profile-PARALOG,ST4:	17 abaumannii(Pasteu	r) ST2	Mbl_NC_010410,Zn-dependent_hydrolase_NC_010410,blaADC-143_NG_064698.1,blaOXA-202_NG_049500.
J414 P	ASS	1	108.2	5411032 1.0597 (.8591)	215 50.3 Escherichia coli	99.97 ANI_match	99.65 ANI_REFSEQ Escherichia(33.70%) coli(29.16%)	Unknown	ecoli(Achtman)	ST11	ecoli_2(Pasteur)	ST628	AmpC1_Ecoli_FN649414,Penicillin_Binding_Protein_Ecoli_CP002291,ampH_Ecoli_AP012030,blaEC-15_NG_0



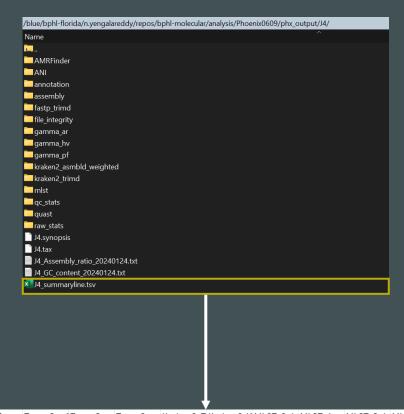


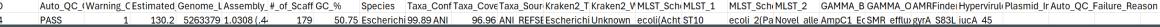
WGS_ID	Parent_Folder Da	ata_Location	Minimum_QC_6	Minimum_QC_	Warnings	Alerts	Raw_Q30_R1_[¹Raw	_Q30_R2_[¹Tot	al_Raw_[rea Pa	ired_Trimmer To	al_Trimmed_Est	imated_Trim GC[%]	Scaffolds	As	sembly_Leng Ass	embly_Ratic Asse	embly_StDe Taxa_Source	Kraken_ID_Raw Kraken_ID_WtA FastANI_Organ F	FastANI_%ID	FastANI_%Cov Species_Suppc Primary_MLST_:	Primary_MLS	ST_: Primary_MLST	Primary_MLST_/ Second	ary_ML! Secondary_	ML: Secondary_ML	LS Secondary_MLS	AR_Database No_
J4	/blue/bphl-flori ph	hx_output	PASS		<70% of read:	is coverage >100:	94.04	86.82	3399434	2946464	3122418	130.18	50.75	179	5263379	1.0308	0.4432 ANI_REFSEQ	Escherichia (14 Escherichia (97 Escherichia col	99.89	96.96 Escherichia_co ecoli(Achtman)	assembly	ST10	adk(10).fumC(:ecoli_2	Pasteu assembly	Novel_allele	dinB(8).icdA(2)	ResGANNCBI_2024
J7	/blue/bphl-flori ph	hx_output	PASS		<70% of read:	is assigned to top	93.41	85.5	2176320	1852286	1976454	80.52	50.46	246	5328404	1.0435	0.6263 ANI_REFSEQ	Escherichia (16 Escherichia (98 Escherichia col	99.95	98.98 Escherichia_co ecoli(Achtman)	assembly	ST21	adk(16).fumC(4 ecoli_2	Pasteu assembly	ST481	dinB(5).icdA(3)	ResGANNCBI_2024
J8	/blue/bphl-flori ph	hx_output	PASS		<70% of read:	is assigned to top	92.44	88.14	2738936	2400072	2528177	100.44	50.31	294	5505983	1.0783	1.1266 ANI_REFSEQ	Escherichia (17 Escherichia (98 Escherichia col	99.66	96.31 Escherichia_co ecoli(Achtman)	assembly	ST29	adk(6).fumC(4) ecoli_2	Pasteu assembly	ST481	dinB(5).icdA(3)	ResGANNCBI_2024
J9	/blue/bphl-flori ph	hx_output	PASS		<70% of reads	ls coverage >100:	93.95	82.47	2631834	2161400	2352199	109.14	50.71	57	4684746	0.9175	1.1868 ANI_REFSEQ	Escherichia (7. Escherichia (99 Escherichia col	99.46	96.09 Escherichia_co ecoli(Achtman)	assembly	ST1248	adk(6).fumC(2! ecoli_2	Pasteul assembly	Novel_allele	dinB(~82).icdA	ResGANNCBI_2024
J17	/blue/bphl-flori ph	hx_output	PASS		<70% of read:	is assigned to top	93.28	85.82	2418606	2071012	2205606	89.77	50.53	267	5350037	1.0478	0.6873 ANI_REFSEQ	Escherichia (15 Escherichia (98 Escherichia col	99.86	99.16 Escherichia_co ecoli(Achtman)	assembly	ST17	adk(6).fumC(4) ecoli_2	Pasteu assembly	Novel_allele	dinB(5).icdA(47)	ResGANNCBI_2024
J36	/blue/bphl-flori ph	hx_output	PASS				90.24	81.23	2528286	2138338	2286558	86.57	57.51	51	5427905	0.9678	0.6836 ANI_REFSEQ	Klebsiella (85.1 Klebsiella (98.3 Klebsiella pneu	99.93	95.57 Klebsiella_pnei kpneumoniae	assembly	ST11	gapA(3).infB(3) -	-	-	-	ResGANNCBI_2024
J40	/blue/bphl-flori ph	hx_output	PASS				90.44	81.31	1588860	1309798	1415918	60.2	55.11	70	4872278	0.9778	0.4291 ANI_REFSEQ	Enterobacter (8 Enterobacter (9 Enterobacter he	99.83	96.17 Enterobacter_h ecloacae	assembly	ST102	dnaA(59).fusA( -	-	-	- 1	ResGANNCBI_2024
J52	/blue/bphl-flori ph	hx_output	PASS		<70% of read:	is coverage >100:	94.64	83.86	3086434	2584824	2791309	119.7	50.45	137	5051000	0.9892	0.155 ANI_REFSEQ	Escherichia (10 Escherichia (98 Escherichia col	99.6	89.31 Escherichia_co ecoli(Achtman)	assembly	ST10	adk(10).fumC(:ecoli_2	Pasteu assembly	ST367	dinB(8).icdA(10)	ResGANNCBI_2024
J100	/blue/bphl-flori ph	hx_output	PASS		<70% of read:	Is assigned to top	93.89	84.75	2153930	1818424	1950807	87.21	50.57	99	4882908	0.9563	0.6285 ANI_REFSEQ	Escherichia (11 Escherichia (98 Escherichia col	99.98	97.98 Escherichia_co ecoli(Achtman)	assembly	ST410	adk(6).fumC(4) ecoli_2	Pasteu assembly	Novel_allele	dinB(6).icdA(6)	ResGANNCBI_2024
J102	/blue/bphl-flori ph	hx_output	PASS				92.05	79.29	2155298	1771506	1925033	69.61	56.81	67	5752511	1.0257	0.5467 ANI_REFSEQ	Klebsiella (80.7 Klebsiella (98.4 Klebsiella pneu	99.95	93.26 Klebsiella_pnet kpneumoniae	assembly	ST307	gapA(4).infB(1) -	-	-	-	ResGANNCBI_2024
J120	/blue/bphl-flori ph	hx_output	PASS		Average Q30 o	of raw R1 reads <9	88.45	76.44	2245352	1895040	2031148	54.22	65.43	128	7316659	1.1005	1.5743 ANI_REFSEQ	Pseudomonas Pseudomonas	99.93	92.23 Pseudomonas_paeruginosa	assembly	ST244	acsA(17).aroE(: -	-	-	-	ResGANNCBI_2024
J217	/blue/bphl-flori ph	hx_output	PASS				97.24	92.38	1750586	1571496	1638543	93.49	39.01	65	3873477	0.9715	0.4391 ANI_REFSEQ	Acinetobacter ( Acinetobacter ( Acinetobacter t	99.87	96.66 Acinetobacter_ abaumannii(Ox	assembly	ST417	gltA(1).gyrB(17 abauma	nnii(Pa assembly	ST2	cpn60(2).fusA(	ResGANNCBI_2024
J414	/blue/bphl-flori ph	hx_output	PASS		<70% of read:	is coverage >100:	93.48	85.19	2956942	2509928	2685208	108.15	50.28	215	5411032	1.0597	0.8591 ANI_REFSEQ	Escherichia (33 Escherichia (98 Escherichia col	99.97	99.65 Escherichia_co ecoli(Achtman)	assembly	ST11	adk(12).fumC(:ecoli_2	Pasteu assembly	ST628	dinB(59).icdA(1	ResGANNCBI_2024
J756	/blue/bphl-flori ph	hx_output	PASS				93.2	76.25	1948216	1470394	1671624	70.98	52.03	73	4971473	1.0273	0.5331 ANI_REFSEQ	Salmonella (91 Salmonella (99 Salmonella enti	99.98	99.07 Salmonella_ent senterica	assembly	ST365	aroC(130).dnal -	-	-	-	ResGANNCBI_2024
J762	/blue/bphl-flori ph	hx_output	FAIL	High scaffold c	ount >500 (92	23).	90.07	85.93	1292508	1112848	1180250	46.55	51.88	923	5429563	1.122	2.3787 ANI_REFSEQ	Salmonella (93 Salmonella (88 Salmonella enti	99.94	98.73 Salmonella_ent senterica	assembly	ST118	aroC(16).dnaN -	-	-		ResGANNCBI_2024
J780	/blue/bphl-flori ph	hx_output	PASS		Average Q30 o	of raw R1 reads <9	89.62	86.62	1614562	1395998	1478162	58.78	51.74	268	5275810	1.0902	1.7593 ANI_REFSEQ	Salmonella (89 Salmonella (97 Salmonella enti	99.64	94.34 Salmonella_ent senterica	assembly	ST964	aroC(13).dnaN -	-	-	-	ResGANNCBI_2024
4																											



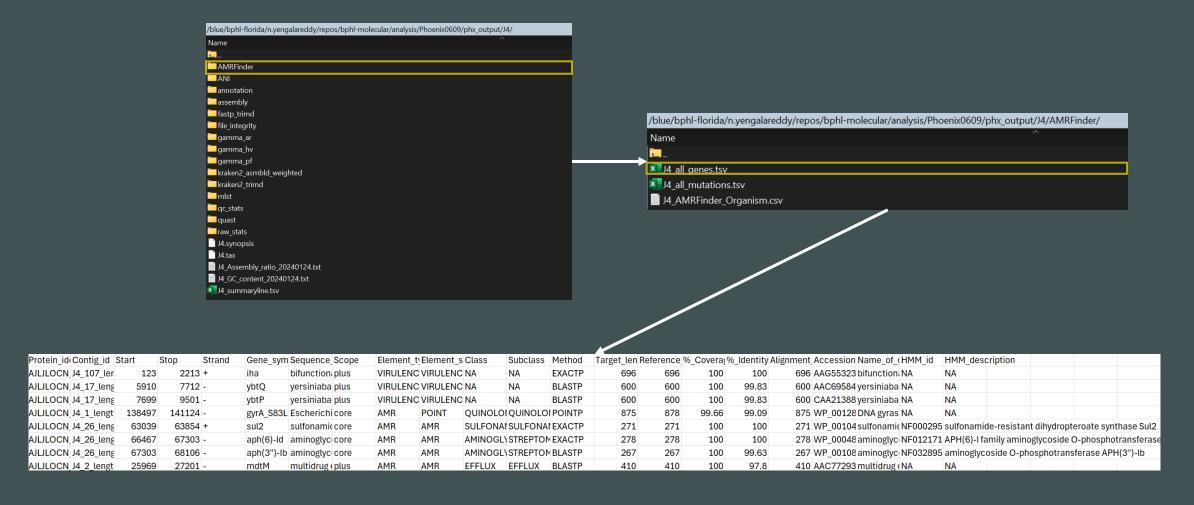




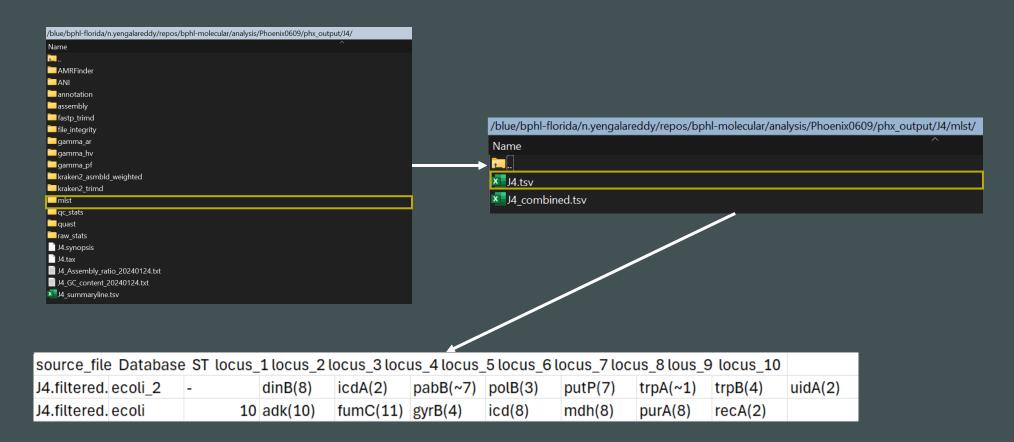






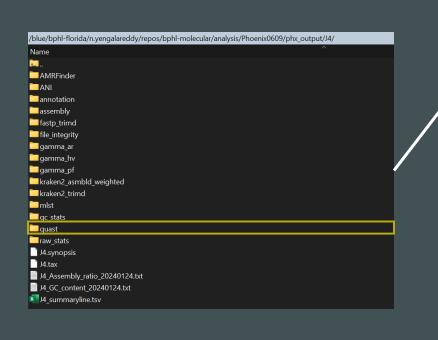


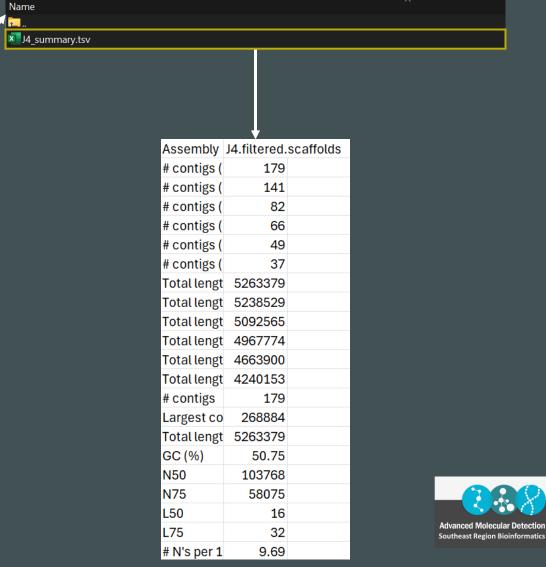






#### Application Cont.





/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Phoenix0609/phx\_output/J4/quast/

#### Conclusion



Fundamentals of PHoeNIx



Installation and setup of PHoeNIx in HPG



Successfully executed job query for PHoeNIx



Generated output files





## **Advanced Molecular Detection**Southeast Region Bioinformatics

**Questions?** 

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Nikhil Reddy, MS
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