### FLAQ- SARS- COV- 2

Advanced Molecular Detection Southeast Region Bioinformatics

November 18, 2024

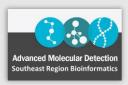
# Bioinformatics Updates From ARLN

#### Candida auris

- MycoSNP v 1.6 (Coming Soon-End of Year):
  - Clade Assignment Functionality
  - Genome Coverage and Depth Metrics
  - Additional Validated Hotspot Regions
  - Downloadable Clade References
- Exploring tools for Long-Sequencing Analysis

#### **PHoeNIx**

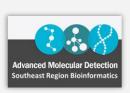
- Phlyophoenix:
  - Seamlessly integrates with Phoenix Workflow
  - Output supports direct upload to Microreact
  - New TSV output includes Epidemiology-Related Metadata



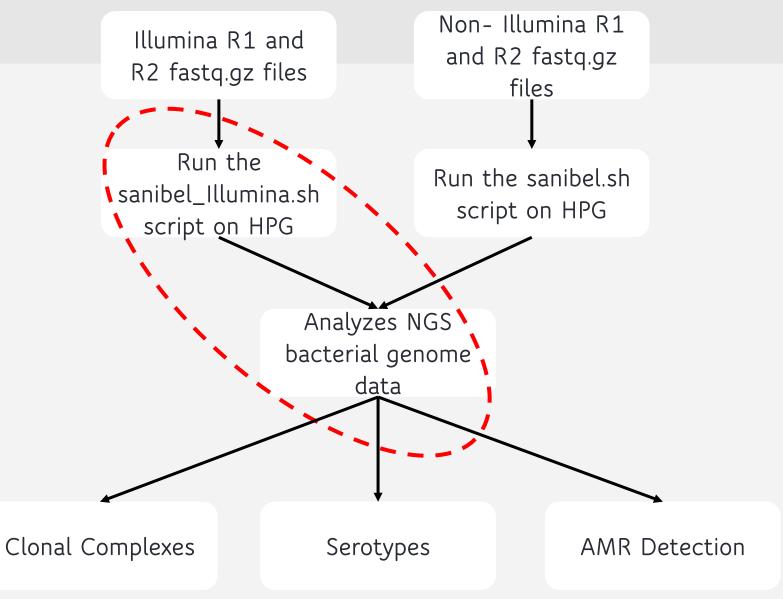
#### Office Hours

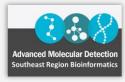
- November 25, 2024 Daytona
- December 09, 2024 FLAQ\_SC2\_Meta

### Office Hours Updates



### Recap of Sanibel





### Sanibel Cont.

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### Sanibel Cont.

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sampleib	speciesiD_masn	nearest_neignb_masn_masr	_distance speciesiD_kraken	kraken_percent mist_scheme	mist_st_mist_cc	pmga_species	serotype	num_clean_reads	avg_readlength	avg_read_quai	est_coverage	num_contigs	iongest_contig	N50	.50 total_i	ngtn gc_conten	t annotated_cds
J100	Escherichia_coli	GCF_000692815.1	0.00492718 Escherichia coli	49.98 ecoli	410			1865832	226.99	37.09	87.27	101	327342	191011	10 48	52648 5	50 4528
J102	Klebsiella_pneumoniae	NZ_CCGO	0.00308338 Klebsiella pneumoniae	81.39 kpneumoniae	307			1810976	219.9	36.79	69.51	74	464840	307140	8 57	29338 5	56 5299
J120	Pseudomonas_aeruginosa	GCF_000629065.1	0.0037263 Pseudomonas aeruginosa	91.74 paeruginosa	244			1917736	211.78	36.36	55.77	130	661600	185918	11 72	82290 6	55 6801
J17	Escherichia_coli	NZ_JHHE	0.00337267 Escherichia coli	57.04 ecoli	17			2119802	226.71	37.06	91.97	244	348576	125279	14 52	25359 5	60 4989
J217	Acinetobacter_baumannii	NZ_AYGJ	0.00393593 Acinetobacter baumannii	76.05 abaumannii_2	2			1590920	226.35	37.74	93.44	68	253267	148812	11 38	53980 3	3598
J36	Klebsiella_pneumoniae	GCF_000445405.1	0.00508714 Klebsiella pneumoniae	83.9 kpneumoniae	11			2181456	218.57	36.64	88.16	69	747923	224752	8 54	08374 5	57 5036
J40	Enterobacter_sp.	GCF_000958405.1	0.0111761 Enterobacter hormaechei	18.68 ecloacae	102			1347996	219.73	36.63	61.04	75	339727	201259	9 48	51991 5	55 4540
J414	Escherichia_coli	NZ_AVRE	0.00243596 Escherichia coli	67 ecoli	11			2575052	226.73	37.08	109.58	189	374706	163749	10 53	27746 5	5062
J4	Escherichia_coli	NZ_JNRV	0.00936594 Escherichia coli	41.03 ecoli	10			3006450	227.56	37.18	131.65	184	267838	101673	16 51	96939 5	50 4921
J52	Escherichia_coli	GCF_000461215.1	0.00680395 Escherichia coli	39.62 ecoli	10			2645448	225.13	37.12	118.98	128	290536	119249	15 50	05322 5	60 4756
J <b>7</b> 56	Salmonella_enterica	GCF_000171495.1	0.00331442 Salmonella enterica	95.86 senterica	365			1530610	221.32	36.85	68.4	76	332968	162252	10 49	52534 5	52 4629
J <b>7</b> 62	Salmonella_enterica	NZ_AJMO	0.00518381 Salmonella enterica	96.64 senterica	118			1144434	225.27	36.75	53.94	40	692679	416934	5 47	79020 5	52 4473
J780	Salmonella_enterica	GCF_000493295.1	0.00737682 Salmonella enterica	93.56 senterica	964			1428284	221.39	36.72	63.81	93	561170	323319	7 49	55382 5	52 4716
J <b>7</b>	Escherichia_coli	NZ_JHGK	0.00243596 Escherichia coli	57.49 ecoli	21			1893390	226.08	37.11	81.91	229	266530	125958	16 52	25938 5	50 5019
J8	Escherichia_coli	NZ_AIBR	0.00638544 Escherichia coli	53.46 ecoli	29			2452914	227.54	37.06	103.7	281	211567	73415	23 53	81948 5	50 5221
J9	Escherichia_coli	GCF_000458955.1	0.00375609 Escherichia coli	51.24 ecoli	1248			2227612	226.32	37.04	108	64	703240	213324	7 46	67970 5	60 4352



#### <u>Purpose</u>

- Analyzes SARS-COV-2 Genomic Data
- Generates Accurate Consensus Assemblies

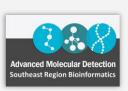
#### <u>Usage</u>

 To support public health and researchers to monitor the spread and evolution of COVID-19

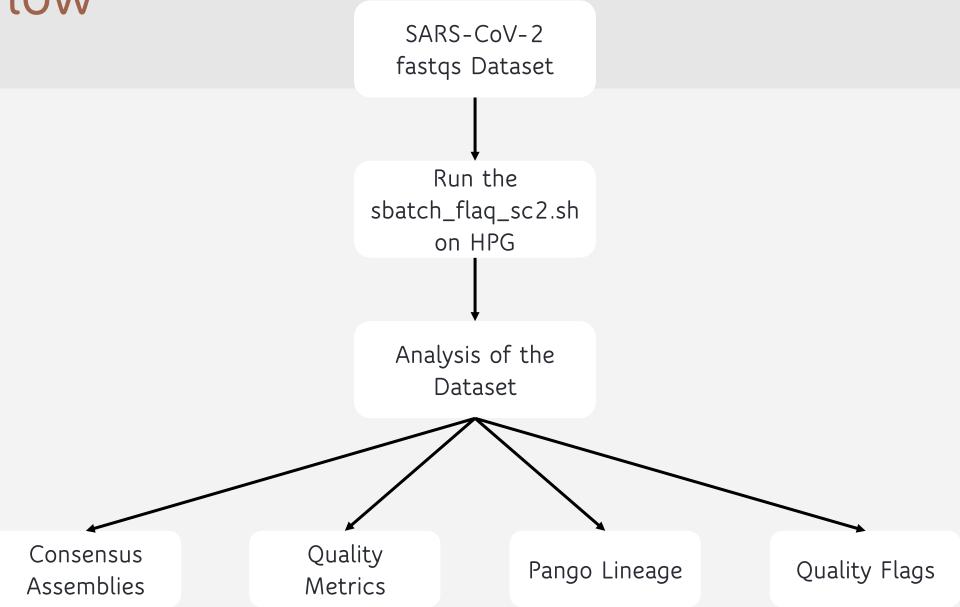
#### <u>Dependencies</u>

- Python 3
- Singularity/Apptainer
- iVar
- Git

### FLAQ-SC2 Overview



#### Workflow





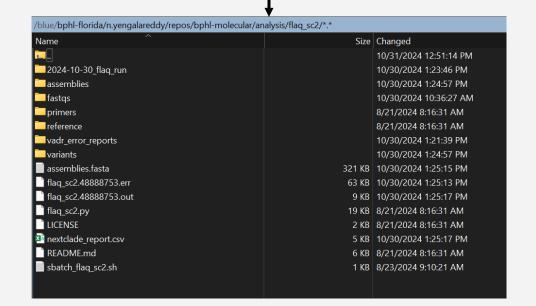
### Application

#### **Objective**

• Use SC2-positive clinical datasets and analyze the dataset using FLAQ-SC2 pipeline.



```
cd /blue/bphl-<state>/<user>/repos/bphl-molecular/
git clone https://github.com/BPHL-Molecular/flaq_sc2
mkdir analysis/
cd analysis/
cp /blue/bphl-<state>/<user>/repos/bphl-
molecular/flaq_sc2/*
mkdir fastqs/
cp /path/to/fastqs/*.fastq.gz fastqs/
```



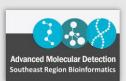


```
nano sbatch_flaq_sc2.sh
```

```
#SBATCH --account=bphl-umbrella
#SBATCH --job-name=flaq_sc2
#SBATCH --job-name=flaq_sc2
#SBATCH --mail-type=END, FAIL
#SBATCH --mail-user=nikhil.yengala@flhealth.gov
#SBATCH --nail-user=nikhil.yengala@flhealth.gov
#SBATCH --ntasks=1
#SBATCH --ntasks=1
#SBATCH --mem=100qb
#SBATCH --time=24:00:00
#SBATCH --time=24:00:00
#SBATCH --output=flaq_sc2.*j.out
#SBATCH --output=flaq_sc2.*j.err
#Run script/command and use $SLURM_CPUS_ON_NODE
module load apptainer
module load apptainer
module load conda
conda activate pyivar

primer_version="4.1"

python flaq_sc2.py fastqs/ --primer_bed primers/ARTIC-V${primer_version}.bed --lib_frag frag --threads $SLURM_CPUS_ON_NODE --r$
```



activate conda environment containing iVar
&
sbatch sbatch\_flaq\_sc2.sh

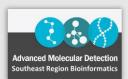
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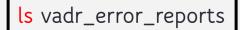




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MN908947.3	3037	7 C	T	r	0	0	0	0 1144	4 433	50	4	1 1144		J TRUE	ORF1ab:cds-YP_009725295.1	TTC	F	TTT	F	924
MN908947.3	14408	3 C	T	1	1	1	76	6 5611	1 3615	47	0.999822	2 5612	<u> </u>	J TRUE	ORF1ab:cds-YP_009724389.1	CCT	P	CTT	L	314
MN908947.3	18877	<i>1</i> C	T		2	1	37	7 2918	8 2036	47	0.999315	5 2920	) (	J TRUE	ORF1ab:cds-YP_009724389.1	CTA	L	TTA	L	1804
MN908947.3	2340?	3 A	G	7	3	2	36	6 5722	2 3527	44	0.999476	6 5725	) (	J TRUE	S:cds-YP_009724390.1	GAT	D	GGT	G	614
MN908947.3	2556?	3 G	T	٢	0	0	0	0 1774	4 882	44	0.999437	7 1775	, (	J TRUE	ORF3a:cds-YP_009724391.1	CAG	Q	CAT	Н	57

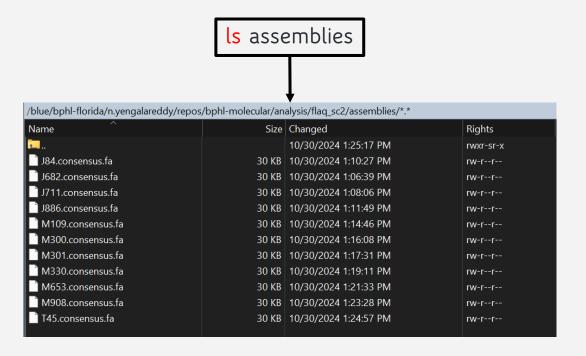




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	_						
	NC_045512			POSSIBLE_FRAMESHIFT		1707021555:+	possible frameshift at 3' end of CDS [length:4482; inserts:none; deletes:S:16943,M:1707017073(4); shifted_frame:3; dominant_frame:1;]
M300	NC_045512	CDS	ORF1ab polyprotein	INDEFINITE_ANNOTATION_END	1694821425:+	2155521555:+	protein-based alignment does not extend close enough to nucleotide-based alignment 3' endpoint [4478>8]
M300	NC_045512	CDS	ORF1ab polyprotein	UNEXPECTED_LENGTH	14013342:+,1334221425:+	26613468:+,1346821555:+	length of complete coding (CDS or mat_peptide) feature is not a multiple of 3 [21287]
M300	NC_045512	mat_peptide	leader protein	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	nsp2	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	nsp3	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	nsp4	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	3C-like proteinase	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	nsp6	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	nsp7	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	nsp8	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	nsp9	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	nsp10	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	RNA-dependent RNA polymerase	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	helicase	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	helicase	UNEXPECTED_LENGTH	1611117909:+	1623718039:+	length of complete coding (CDS or mat_peptide) feature is not a multiple of 3 [1799]
M300	NC_045512	mat_peptide	3'-to-5' exonuclease	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	endoRNAse	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]
M300	NC_045512	mat_peptide	2'-O-ribose methyltransferase	PEPTIDE_TRANSLATION_PROBLEM	-	-	mat_peptide may not be translated because its parent CDS has a problem [-]



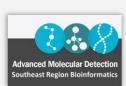




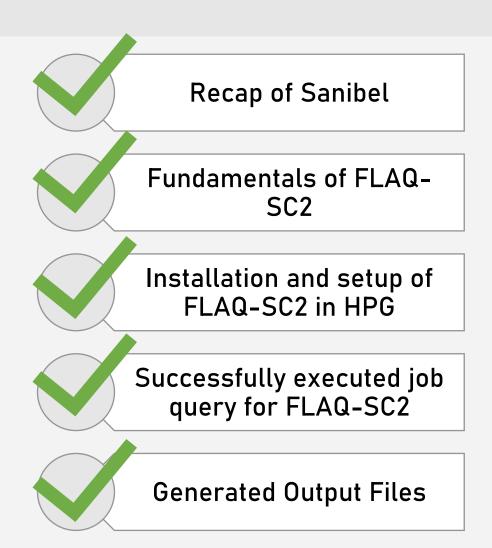


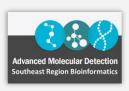
Name	Size	Changed	Rights
<u></u>		10/30/2024 1:25:17 PM	rwxr-sr-x
J84		10/30/2024 1:10:19 PM	rwxr-sr-x
<b>1</b> 682		10/30/2024 1:06:38 PM	rwxr-sr-x
J711		10/30/2024 1:08:04 PM	rwxr-sr-x
<b>1</b> 3886		10/30/2024 1:11:48 PM	rwxr-sr-x
M109		10/30/2024 1:14:37 PM	rwxr-sr-x
M300		10/30/2024 1:16:06 PM	rwxr-sr-x
M301		10/30/2024 1:17:30 PM	rwxr-sr-x
M330		10/30/2024 1:19:08 PM	rwxr-sr-x
M653		10/30/2024 1:21:28 PM	rwxr-sr-x
<b>™</b> M908		10/30/2024 1:23:24 PM	rwxr-sr-x
T45		10/30/2024 1:24:55 PM	rwxr-sr-x
report.txt	2 KB	10/30/2024 1:25:15 PM	rw-rr

sampleID	reference	start end	num_raw_reads	num_clean_reads	num_mapped_reads p	percent_mapped_clean_reads	cov_bases_mapped	percent_genome_cov_map	mean_depth r	nean_base_qual	mean_map_qual	assembly_length	numN	percent_ref_genome_cov_VADR_f	flag QC_fla	g pangolin_version lir	neage SOTC
J682	MN908947.3	1 29903	176476	125270	71227	56.8588	29497	98.6423	503.937	37.8	60	29777	1310	95.1978 PASS	PASS	v4.3.1_pdata-v1.29 B.	.1
J711	MN908947.3	1 29903	442888	279008	167235	59.9391	29818	99.7157	776.595	35.6	60	29829	650	97.5788 PASS	PASS	v4.3.1_pdata-v1.29 B.	.1
J84	MN908947.3	1 29903	733132	606216	458864	75.6932	29868	99.883	3602.92	37.7	60	29862	50	99.6957 PASS	PASS	v4.3.1_pdata-v1.29 B.	.1
J886	MN908947.3	1 29903	467264	295070	143787	48.7298	29634	99.1004	673.339	35.6	60	29777	1125	95.8165 PASS	PASS	v4.3.1_pdata-v1.29 B.	.1
M109	MN908947.3	1 29903	1062220	898804	550019	61.1945	29858	99.8495	4225.17	37.7	60	29858	115	99.4649 PASS	PASS	v4.3.1_pdata-v1.29 B.	.1
M300	MN908947.3	1 29903	289620	252704	121936	48.2525	29718	99.3813	878.313	37.8	60	29773	1439	94.753 REVIEW	/ PASS	v4.3.1_pdata-v1.29 B.	.1
M301	MN908947.3	1 29903	447296	349038	67383	19.3053	29585	98.9366	494.466	37.8	60	29754	3041	89.3322 REVIEW	/ PASS	v4.3.1_pdata-v1.29 B.	.1
M330	MN908947.3	1 29903	407520	363380	169527	46.6528	29756	99.5084	1226.17	37.8	60	29774	726	97.1408 PASS	PASS	v4.3.1_pdata-v1.29 B.	.1
M653	MN908947.3	1 29903	872474	758820	333828	43.993	29825	99.7392	2292.56	37.8	60	29813	664	97.4785 REVIEW	/ PASS	v4.3.1_pdata-v1.29 B.	.1
M908	MN908947.3	1 29903	530524	467348	253716	54.2885	29807	99.679	1836.15	37.8	60	29807	90	99.378 PASS	PASS	v4.3.1_pdata-v1.29 B.	.1
T45	MN908947.3	1 29903	442412	340932	130766	38.3554	29777	99.5786	757.439	37.7	60	29777	1370	94.9972 PASS	PASS	v4.3.1 pdata-v1.29 B.	.1



#### Conclusion







### Advanced Molecular Detection Southeast Region Bioinformatics

**Questions?** 

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