## Daytona

Advanced Molecular Detection Southeast Region Bioinformatics

November 25, 2024

# A

#### Office Hours

- December 09, 2024 FLAQ\_SC2\_Meta
- December 23, 2024 Daytona\_Dengue



#### Overview

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

Nextflow version of the FLAQ\_SC2 pipeline + Added function of human read removal is added

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

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

#### **Dependencies**

Nextflow
Python3
Singularity

Docker Container Images

1. fastqc\_0.11.9.sif 2. trimmomatic\_0.39.sif

3. bbtools\_38.76.sif 4. multiqc\_1.8.sif

5. bwa\_0.7.17.sif 6. samtools\_1.12.sif

7. vadr\_1.3.sif

8. pangolin\_4.1.2-pdata-1.13.sif

9. nextclade\_2021-03-15.sif

10. sra-human-scrubber\_1.1.2021-05-05.sif



#### Workflow

SARS-CoV-2 fastqs Dataset

Run the sbatch\_flaq\_sc2\_nf.sh on HPG

Analysis of the Dataset

Consensus Assemblies Quality Metrics

Pango Lineage Quality Flags



## Application

#### **Objective**

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



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

git clone https://github.com/BPHL-Molecular/Daytona

mkdir analysis/

cd analysis/

cp /blue/bphl-<state>/<user>/repos/bphlmolecular/Daytona/\*

mkdir fastqs/

cp /path/to/fastqs/\*.fastq.gz fastqs/

	/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/ana	lysis/Daytona1107/*.*	
	Name	Size	Changed
	<u>=</u>		11/7/2024 9:18:47 AM
	apps		11/7/2024 11:06:53 AM
	<b>a</b> fastqs		11/7/2024 9:19:57 AM
	output		11/12/2024 9:16:17 AM
	primers		11/6/2024 1:01:00 PM
	reference		11/6/2024 1:01:00 PM
	work		11/12/2024 9:15:01 AM
<b>→</b>	flaq_sc2_humanclean.nf	18 KB	11/6/2024 1:01:00 PM
	flaq_sc2_humanclean2.nf	19 KB	11/6/2024 1:01:00 PM
	flaq_sc2_nf.49628631.err	0 KB	11/12/2024 9:09:43 AM
	flaq_sc2_nf.49628631.out	52 KB	11/12/2024 9:16:17 AM
	flaq_sc2_nf.nf	18 KB	11/6/2024 1:01:00 PM
	LICENSE	2 KB	11/6/2024 1:00:58 PM
	nextflow	18 KB	11/8/2024 3:01:12 PM
	params.yaml	1 KB	11/7/2024 9:37:31 AM
	README.md	2 KB	11/6/2024 1:00:58 PM
	sbatch_flaq_sc2_nf.sh	2 KB	11/12/2024 9:08:50 AM



/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/apps/staphb-toolkit/containers/									
Name	Size	Changed							
<u> </u>		11/8/2024 2:50:09 PM							
bbtools_38.76.sif	506,204 KB	10/19/2020 8:29:15 AM							
bwa_0.7.17.sif	98,040 KB	10/19/2020 8:30:24 AM							
fastqc_0.11.9.sif	167,016 KB	12/16/2021 12:58:43 PM							
multiqc_1.8.sif	1,266,688 KB	10/18/2020 9:06:46 AM							
nextclade_2021-03-15.sif	323,820 KB	3/15/2021 11:30:42 AM							
pangolin_4.1.2-pdata-1.13.sif	710,076 KB	8/23/2022 7:29:35 AM							
samtools_1.12.sif	117,248 KB	4/14/2021 6:55:50 AM							
sra-human-scrubber_1.1.2021-05-05.sif	1,519,872 KB	8/30/2022 11:12:28 AM							
trimmomatic_0.39.sif	197,320 KB	10/18/2020 9:03:45 AM							
vadr_1.3.sif	727,704 KB	9/6/2021 7:19:16 AM							



/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/ana	lysis/Daytona1107/*.*	
Name	Size	Changed
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primers primers		11/6/2024 1:01:00 PM
reference		11/6/2024 1:01:00 PM
work		11/12/2024 9:15:01 AM
flaq_sc2_humanclean.nf	18 KB	11/6/2024 1:01:00 PM
flaq_sc2_humanclean2.nf	19 KB	11/6/2024 1:01:00 PM
flaq_sc2_nf.49628631.err	0 KB	11/12/2024 9:09:43 AM
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sbatch_flaq_sc2_nf.sh	2 KB	11/12/2024 9:08:50 AM

#### Copy Apps From:

/apps/staphb-toolkit/containers

Ог

ncbi/sra-human-scrubber & StaPH-B/docker-builds

nano params.yaml

params.yaml

```
# The parameters "input", "output", "reference" and "primer" are the absolute paths. Do not include the "/" at the end of the paths.
input : "/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/fastqs"
output : "/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/output"
reference : "/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/reference"
primer : "/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/primers"
# specify if input amplicons were fragmented. If yes, the parameter setting is "frag". If no, the parameter setting is "no_frag". (default: frag)
frag : "no_frag"
# comma separated list of SOTCs to screen, default as "S:L452R,S:E484K"
sotc : "S:L452R,S:E484K"
```



GNU nano 2.9.8

nano sbatch\_flaq\_sc2\_nf.sh

```
GNU nano 2.9.8
                                                                                        sbatch flag sc2 nf.sh
#!/bin/bash
#SBATCH --account=bphl-umbrella
#SBATCH --iob-name=flag sc2 nf
#SBATCH --mail-type=END,FAIL
  BATCH --time=24:00:00
  BATCH --output=flaq_sc2_nf.%j.out
SBATCH --error=flag sc2 nf.%j.err
#Run script/command and use $SLURM CPUS ON NODE
module load apptainer
nextflow run flaq sc2 humanclean2.nf -params-file params.yaml
 sort ./output/*/report.txt | uniq > ./output/sum_report.txt
 ed -i '/sampleID\treference/d' ./output/sum report.txt
 ed -i 'li sampleID\treference\tstart\tend\tnum raw reads\tnum clean reads\tnum mapped reads\tpercent mapped clean reads\tcov bases mapped\tpercent genome cov map\tmean depth\tmean base qual
 at ./output/assemblies/*.fa > ./output/assemblies.fasta
singularity exec /apps/staphb-toolkit/containers/nextclade 2021-03-15.sif nextclade --input-fasta ./output/assemblies.fasta --output-csv ./output/nextclade report.csv
```

activate conda environment containing Nextflow &

sbatch sbatch\_flaq\_sc2\_nf.sh

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/*.*								
Name	Size	Changed						
<u>≔</u>		11/7/2024 9:18:47 AM						
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README.md	2 KB	11/6/2024 1:00:58 PM						
sbatch_flaq_sc2_nf.sh	2 KB	11/12/2024 9:08:50 AM						

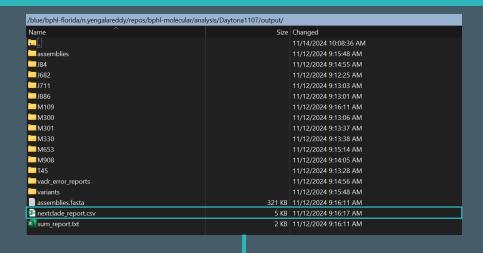


Name ^	Size	Changed	
		11/14/2024 10:08:36 AM	
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■J682		11/12/2024 9:12:25 AM	
<b>□</b> J711		11/12/2024 9:13:03 AM	
■J886		11/12/2024 9:13:01 AM	
■M109		11/12/2024 9:16:11 AM	
■M300		11/12/2024 9:13:06 AM	
M301		11/12/2024 9:13:37 AM	
M330		11/12/2024 9:13:38 AM	
■M653		11/12/2024 9:15:14 AM	
■M908		11/12/2024 9:14:05 AM	
■T45		11/12/2024 9:13:28 AM	
vadr_error_reports		11/12/2024 9:14:56 AM	
■ variants		11/12/2024 9:15:48 AM	
assemblies.fasta	321 KB	11/12/2024 9:16:11 AM	
nextclade_report.csv	5 KB	11/12/2024 9·16·17 AM	
sum_report.txt	2 KB	11/12/2024 9:16:11 AM	

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Name -	Size Changed
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<b>T</b> fastqs	11/7/2024 9:19:57 AM
output	11/12/2024 9:16:17 AM
primers	11/6/2024 1:01:00 PM
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flaq_sc2_humanclean2.nf	19 KB 11/6/2024 1:01:00 PM
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flaq_sc2_nf.49628631.out	52 KB 11/12/2024 9:16:17 AM
flaq_sc2_nf.nf	18 KB 11/6/2024 1:01:00 PM
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README.md	2 KB 11/6/2024 1:00:58 PM
sbatch flag sc2 nf.sh	2 KB 11/12/2024 9:08:50 AM

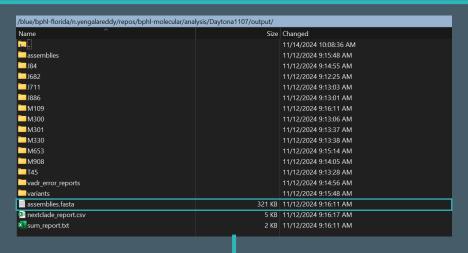
sampleID	reference	start end	num_raw_reads r	num_clean_reads	num_mapped_reads	percent_mapped_clean_reads c	ov_bases_mapped perd	cent_genome_cov_map mea	n_depth	mean_base_qual	mean_map_qual	assembly_length	numN	percent_ref_genome_cov	VADR_fla	g QC_flag	pangolin_version	lineage	OTC
J682	MN908947.3	1 29903	176476	125270	105571	84.2748	29526	98.7393	756.939	37.8	60	2980	1 1317	95.2547	PASS	PASS	v4.1.2_pdata-v1.13	B.1	
J711	MN908947.3	1 29903	442888	279008	277991	99.6355	29830	99.7559	1276.76	35.5	60	2984	1 673	97.5421	PASS	PASS	v4.1.2_pdata-v1.13	B.1	
J84	MN908947.3	1 29903	733132	606216	595444	98.2231	29849	99.8194	4674.64	37.7	60	2984	3 18	99.7392	PASS	PASS	v4.1.2_pdata-v1.13	B.1	
J886	MN908947.3	1 29903	467264	295070	285981	96.9197	29664	99.2007	1319.11	35.5	60	2980	2 1126	95.8967	PASS	PASS	v4.1.2_pdata-v1.13	B.1	
M109	MN908947.3	1 29903	1062220	898804	878602	97.7523	29858	99.8495	6765.29	37.7	60	2985	8 105	99.4984	PASS	PASS	v4.1.2_pdata-v1.13	B.1	
M300	MN908947.3	1 29903	289620	252704	240562	95.1952	29703	99.3312	1784.04	37.7	60	2975	8 1392	94.86	REVIEW	PASS	v4.1.2_pdata-v1.13	B.1	
M301	MN908947.3	1 29903	447296	349038	119960	34.3688	29606	99.0068	900.226	37.7	60	2975	9 2959	89.6231	REVIEW	PASS	v4.1.2_pdata-v1.13	B.1.124	
M330	MN908947.3	1 29903	407520	363380	359808	99.017	29741	99.4582	2692.15	37.8	60	2975	9 688	97.2177	PASS	PASS	v4.1.2_pdata-v1.13	B.1	
M653	MN908947.3	1 29903	872474	758820	754273	99.4008	29838	99.7826	5429.88	37.7	60	2982	5 634	97.619	REVIEW	PASS	v4.1.2_pdata-v1.13	B.1	
M908	MN908947.3	1 29903	530524	467348	467739	100.0837	29802	99.6622	3472.95	37.7	60	2980	2 91	99.3579	PASS	PASS	v4.1.2_pdata-v1.13	B.1	
T45	MN908947.3	1 29903	442412	340932	246111	72.1877	29801	99.6589	1443.61	37.7	60	2980	1 1427	94.8868	PASS	PASS	v4.1.2 pdata-v1.13	B.1	





segName:clade:gc.overallScore:gc.overallScore:gc.overallStatus:totalGaps:totalInsertions:totalMissing:totalMissing:totalMissing:totalMutations:totalMunations:totalMissing.tot





#### >J682



lame ^	Size Changed
	11/12/2024 9:16:17 AM
J84.variants.tsv	2 KB 11/12/2024 9:14:32 AM
J682.variants.tsv	2 KB 11/12/2024 9:11:47 AM
J711.variants.tsv	1 KB 11/12/2024 9:12:25 AM
J886.variants.tsv	1 KB   11/12/2024 9:12:25 AM
M109.variants.tsv	1 KB 11/12/2024 9:15:48 AM
M300.variants.tsv	2 KB 11/12/2024 9:12:25 AM
M301.variants.tsv	2 KB 11/12/2024 9:13:02 AM
M330.variants.tsv	1 KB 11/12/2024 9:13:02 AM
M653.variants.tsv	2 KB 11/12/2024 9:14:47 AM
M908.variants.tsv	1 KB 11/12/2024 9:13:39 AM
T45.variants.tsv	1 KB 11/12/2024 9:12:55 AM

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/output/								
Name	Size	Changed						
		11/14/2024 10:08:36 AM						
assemblies		11/12/2024 9:15:48 AM						
<b>□</b> J84		11/12/2024 9:14:55 AM						
□J682		11/12/2024 9:12:25 AM						
<b>□</b> J711		11/12/2024 9:13:03 AM						
<b>□</b> J886		11/12/2024 9:13:01 AM						
<b>™</b> M109		11/12/2024 9:16:11 AM						
<u>™</u> M300		11/12/2024 9:13:06 AM						
™301		11/12/2024 9:13:37 AM						
™330		11/12/2024 9:13:38 AM						
™653		11/12/2024 9:15:14 AM						
<u>™</u> M908		11/12/2024 9:14:05 AM						
T45		11/12/2024 9:13:28 AM						
vadr_error_reports		11/12/2024 9:14:56 AM						
□ variants		11/12/2024 9:15:48 AM						
assemblies.fasta	321 KB	11/12/2024 9:16:11 AM						
nextclade_report.csv	5 KB	11/12/2024 9:16:17 AM						
sum_report.txt	2 KB	11/12/2024 9:16:11 AM						

REGION	POS	REF	ALT	REF_DP	REF_RV R	EF_QUAL	ALT_DP	ALT_RV	ALT_QUAL	ALT_FREQ	TOTAL_DP	PVAL	PASS	GFF_FEATURE	REF_CODON	REF_AA	ALT_CODON	ALT_AA	POS_AA
MN908947.3	241	C	Т	C	0	0	436	29	48	1	436	1.36E-296	TRUE	NA	NA	NA	NA	NA	NA
MN908947.3	514	Т	#NAME?	1070	185	48	1332	0	20	0.909215	1465	0	TRUE	NA	NA	NA	NA	NA	NA
MN908947.3	520	G	Т	2	2 0	76	8	3	34	0.8	10	8.60E-19	TRUE	ORF1ab:cds-YP_009724389.1	ATG	M	ATT	I	85
MN908947.3	520	G	Т	2	0	76	8	3	34	0.8	10	8.60E-19	TRUE	ORF1ab:cds-YP_009725295.1	ATG	M	ATT	I	85
MN908947.3	3037	С	Т	C	0	0	1252	265	49	1	1252	0	TRUE	ORF1ab:cds-YP_009724389.1	TTC	F	TTT	F	924
MN908947.3	3037	С	Т	C	0	0	1252	265	49	1	1252	0	TRUE	ORF1ab:cds-YP_009725295.1	TTC	F	TTT	F	924
MN908947.3	14408	С	Т	2	0	57	5933	2609	45	0.999495	5936	0	TRUE	ORF1ab:cds-YP_009724389.1	CCT	P	CTT	L	314
MN908947.3	18877	С	Т	3	1	37	3607	2082	47	0.999169	3610	0	TRUE	ORF1ab:cds-YP_009724389.1	CTA	L	TTA	L	1804
MN908947.3	23403	Α	G	5	3	44	6298	3566	42	0.999207	6303	0	TRUE	S:cds-YP_009724390.1	GAT	D	GGT	G	614
MN908947.3	25563	G	T	C	0	0	2166	945	44	0.999539	2167	0	TRUE	ORF3a:cds-YP_009724391.1	CAG	Q	CAT	Н	57



/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/output/vadr_error_reports/									
Name	Size	Changed							
<mark>≔</mark>		11/12/2024 9:16:17 AM							
M300.vadr.alt.list	4 KB	11/12/2024 9:12:37 AM							
M301.vadr.alt.list	4 KB	11/12/2024 9:13:12 AM							
M653.vadr.alt.list	1 KB	11/12/2024 9:14:56 AM							

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Daytona1107/output/								
Name	Size	Changed						
		11/14/2024 10:08:36 AM						
assemblies assemblies		11/12/2024 9:15:48 AM						
<u></u> J84		11/12/2024 9:14:55 AM						
<u></u> 1682		11/12/2024 9:12:25 AM						
<b>□</b> J711		11/12/2024 9:13:03 AM						
<u></u> 1886		11/12/2024 9:13:01 AM						
<b>™</b> M109		11/12/2024 9:16:11 AM						
<b>™</b> M300		11/12/2024 9:13:06 AM						
<u>™</u> M301		11/12/2024 9:13:37 AM						
<b>™</b> M330		11/12/2024 9:13:38 AM						
M653		11/12/2024 9:15:14 AM						
M908		11/12/2024 9:14:05 AM						
T45		11/12/2024 9:13:28 AM						
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sum_report.txt	2 KB	11/12/2024 9:16:11 AM						

#sequence	model	feature-type	feature-name	error	seq-coords	mdl-coords	error-description
M300	NC_045512	CDS	ORF1ab polyprotein	CDS_HAS_STOP_CODON	1699016992:+	1712017122:+	in-frame stop codon exists 5' of stop position predicted by homology to reference [TAG, shifted S:4433,M:4433]
M300	NC_045512	CDS	ORF1ab polyprotein	POSSIBLE_FRAMESHIFT	1694421425:+	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 [-]

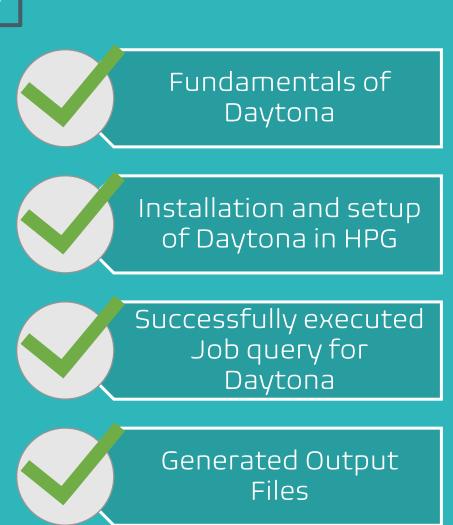
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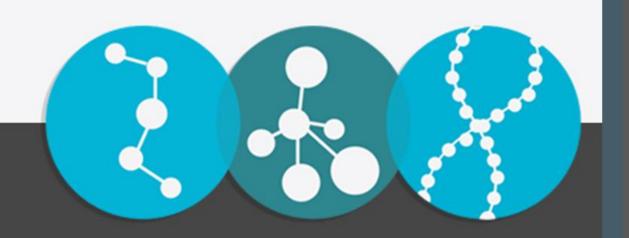
#### >J84



#### Conclusion







# Advanced Molecular Detection Southeast Region Bioinformatics

Questions?

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