03 Feb 2025

Pensacola

Advanced Molecular Detection

Southeast Region Bioinformatics

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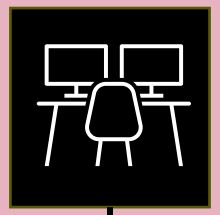
Updates

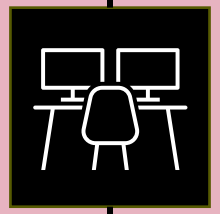
Office Hours

2025



February 17
Talbot

March 03
Sarek_mic



2025

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Overview

Purpose

- Analyze *Candida auris* long-read sequences data for Quality Reports, Genome Assembly, Species Identification and Abundance, SNP analysis, Antifungal Resistance reports, and Phylogenetic analysis.

Usage

- To support public health and researchers by providing detailed reports and analyses of the data which enables insights into drug resistance monitoring, genomic research, outbreak surveillance and epidemiological studies.

Dependencies

- Nextflow
- Singularity/Apptainer
- SLURM
- Python3
- LongQC
- PacBio SMRTLINK
- Kraken2/Bracken

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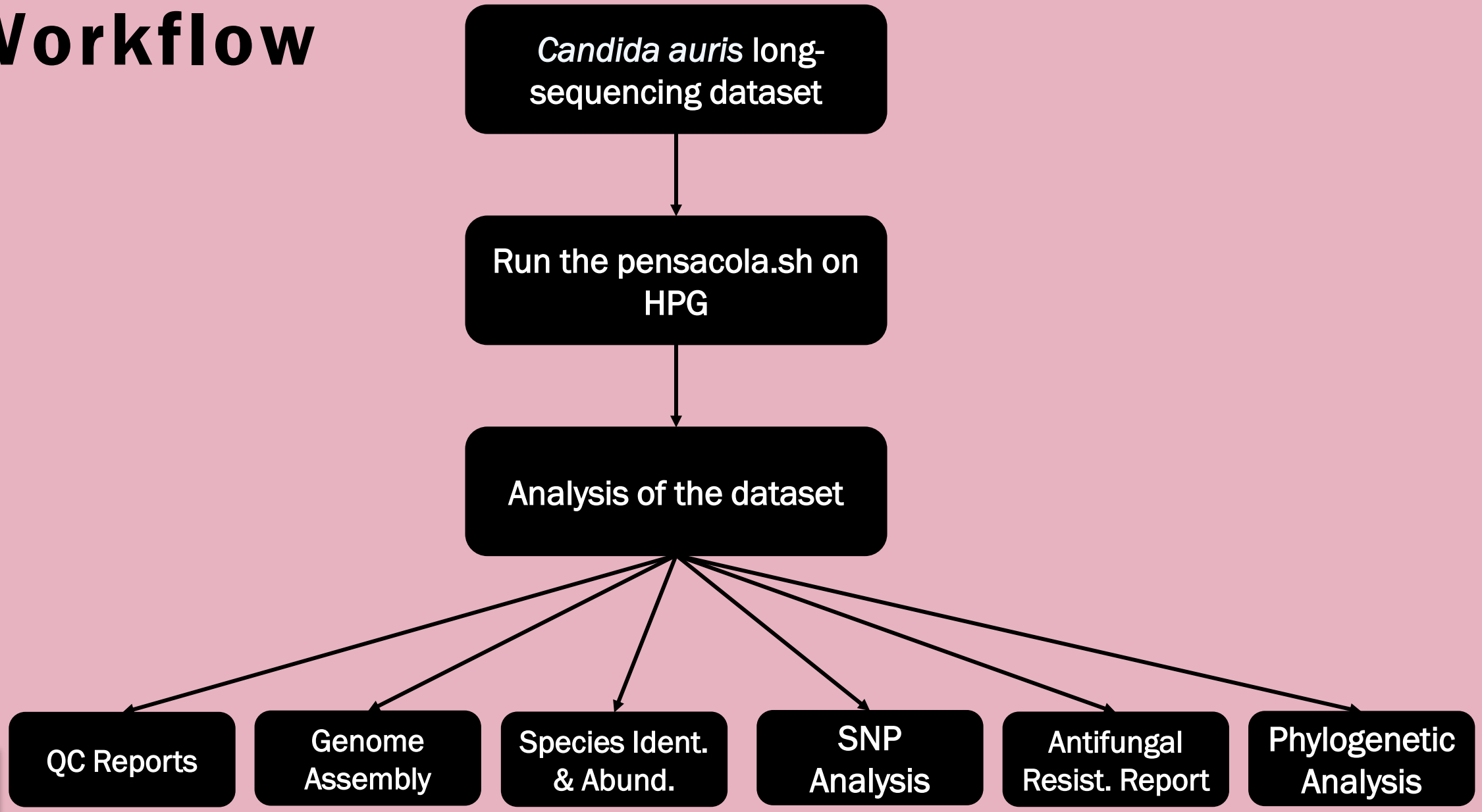
Workflow



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Application

Objective

Use *Candida auris* dataset and analyze using **Pensacola** pipeline

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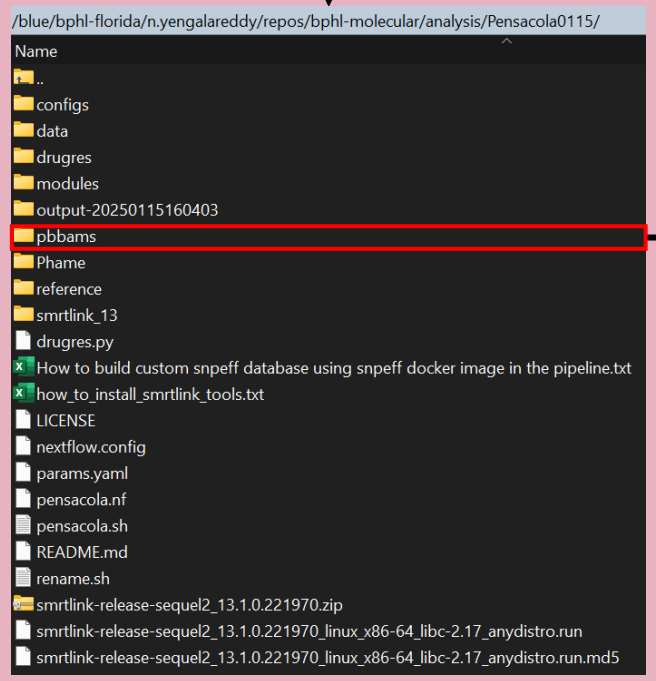
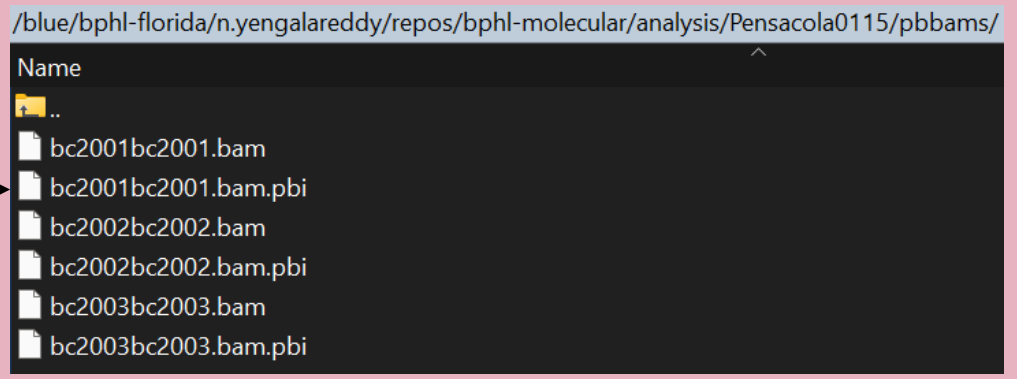
Application Cont.

```
cd /blue/bphl-<state>/<user>/repos/bphl-molecular/
git clone https://github.com/BPHL-Molecular/Pensacola
mkdir analysis/
cd analysis/
cp /blue/bphl-<state>/<user>/repos/bphl-molecular/ Pensacola/
copy .bam and .bam.pbi files to pbbams directory
```





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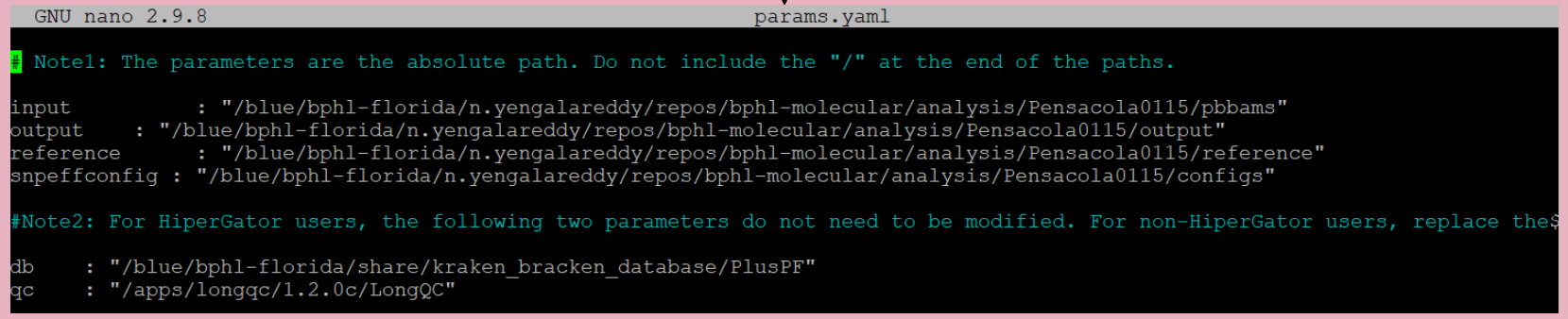
Application Cont.

nano params.yaml



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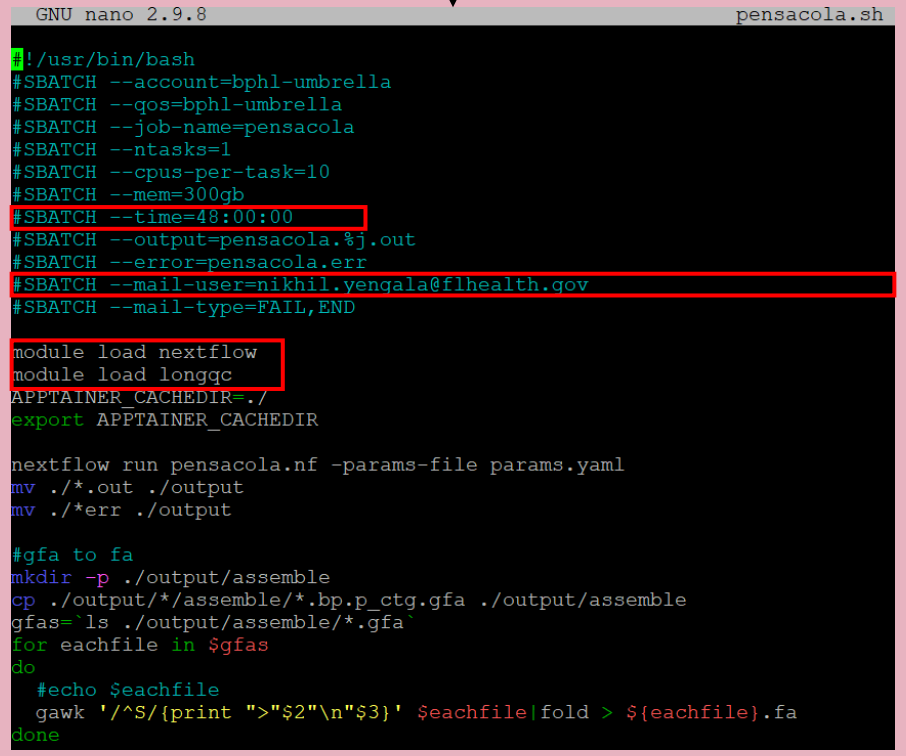
Application Cont.

nano pensacola.sh



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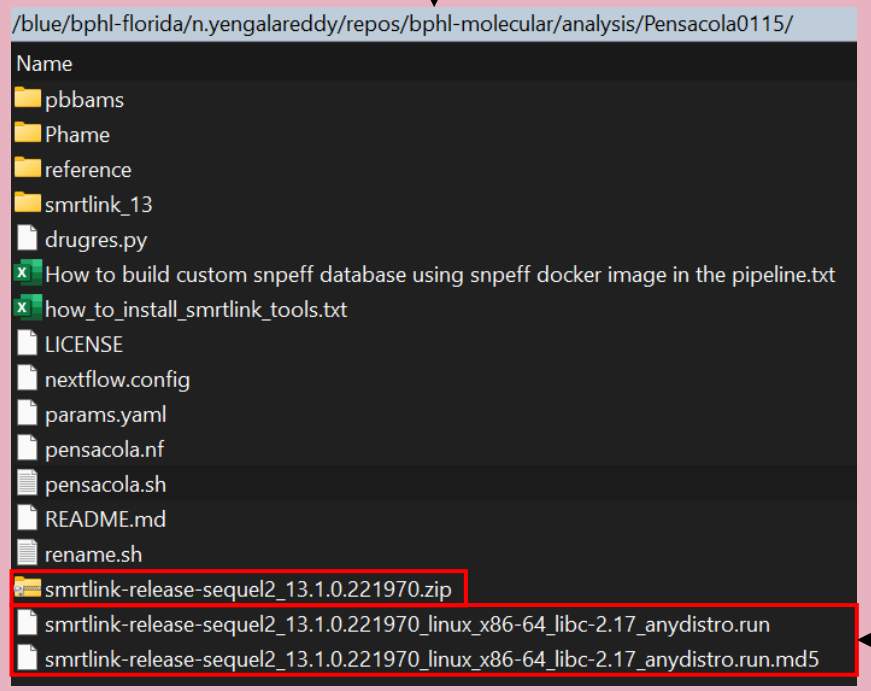
Application Cont.

1) wget https://downloads.pacbcloud.com/public/software/installers/smrtlink-release-sequel2_13.1.0.221970.zip



2) ./smrtlink-release-sequel2_13.1.0.221970.run –rootdir ./smrtlink_13 –smrttools-only

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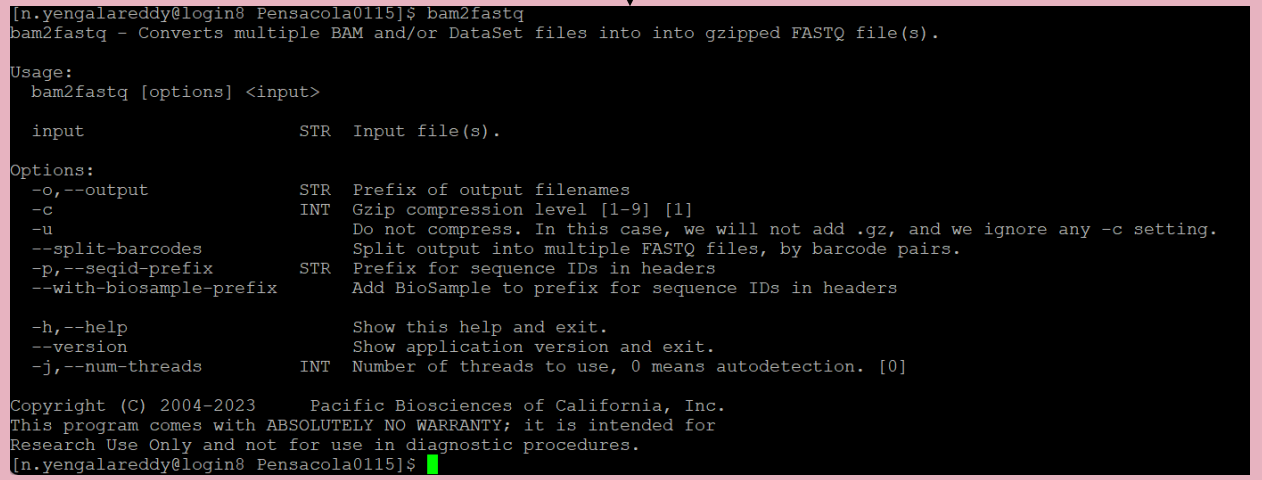
Application Cont.

Note: Double check that smartlink tools works by entering

"bam2fastq" into the command line



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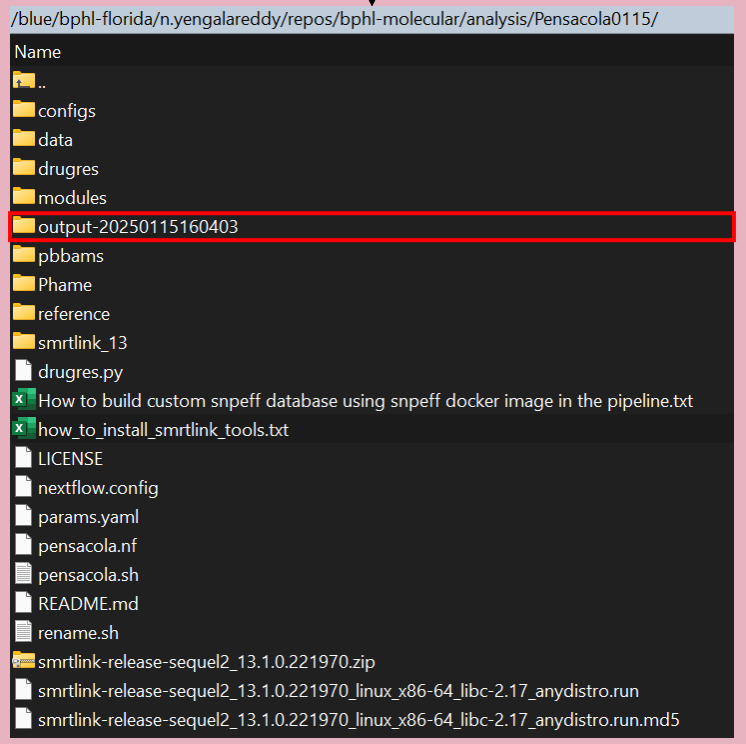
Application Cont.

activate conda environment containing Nextflow, Python3

sbatch pensacola.sh



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Application Cont.

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Pensacola0115/

Name
- ..
- configs
- data
- drugres
- modules
- output-20250115160403
- pbbams
- Phame
- reference
- smrtlink_13
- drugres.py
- How to build custom snpeff database using snpeff docker image in the pipeline.txt
- how_to_install_smrtlink_tools.txt
- LICENSE
- nextflow.config
- params.yaml
- pensacola.nf
- pensacola.sh
- README.md
- rename.sh
- smrtlink-release-sequel2_13.1.0.221970.zip
- smrtlink-release-sequel2_13.1.0.221970_linux_x86-64_libc-2.17_anydistro.run
- smrtlink-release-sequel2_13.1.0.221970_linux_x86-64_libc-2.17_anydistro.run.md5

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Pensacola0115/output-20250115160403/

Name
- ..
- assemble
- bc2001bc2001
- bc2002bc2002
- bc2003bc2003
- species_abundance
- variants
- Drug_resistance_report.txt
- pensacola.55938781.out
- pensacola.err

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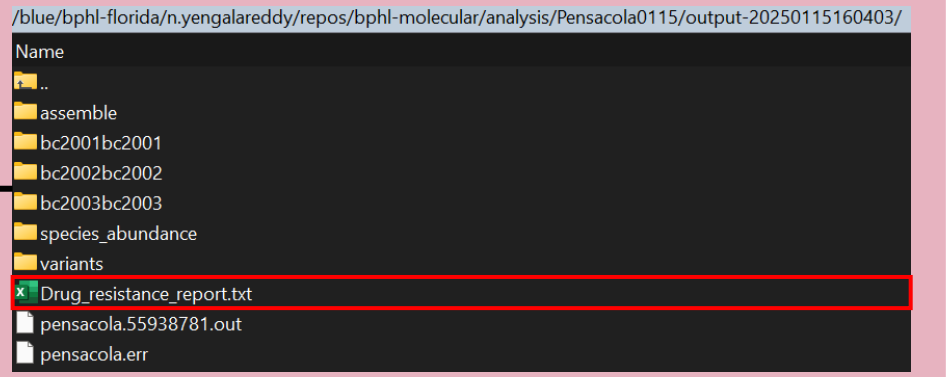
Application Cont.



########## Detected drug resistance ##########

1. bc2001bc2001.variants_snpeff.ann.vcf:

ID,Drug,Gene,Mutation
B9J08_001448,FLC,ERG11,Y132F;K143R;Phe126Leu

B9J08_005576,FLC,,Cys110Phe

2. bc2003bc2003.variants_snpeff.ann.vcf:

ID,Drug,Gene,Mutation
B9J08_001448,FLC,ERG11,Y132F;K143R;Phe126Leu

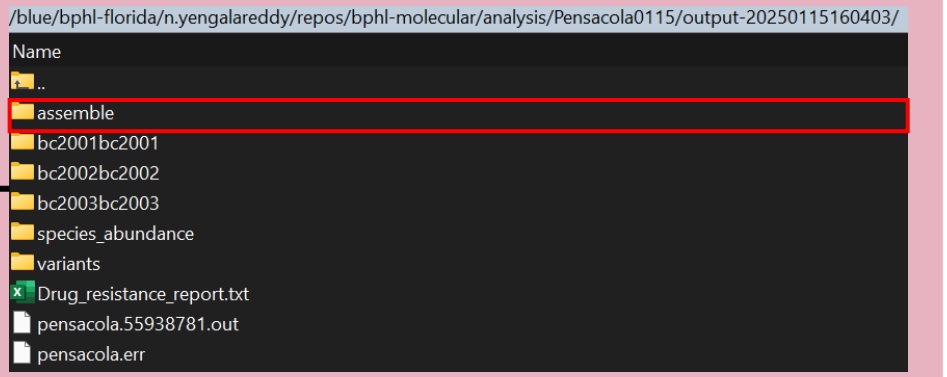
B9J08_005576,FLC,,Cys110Phe

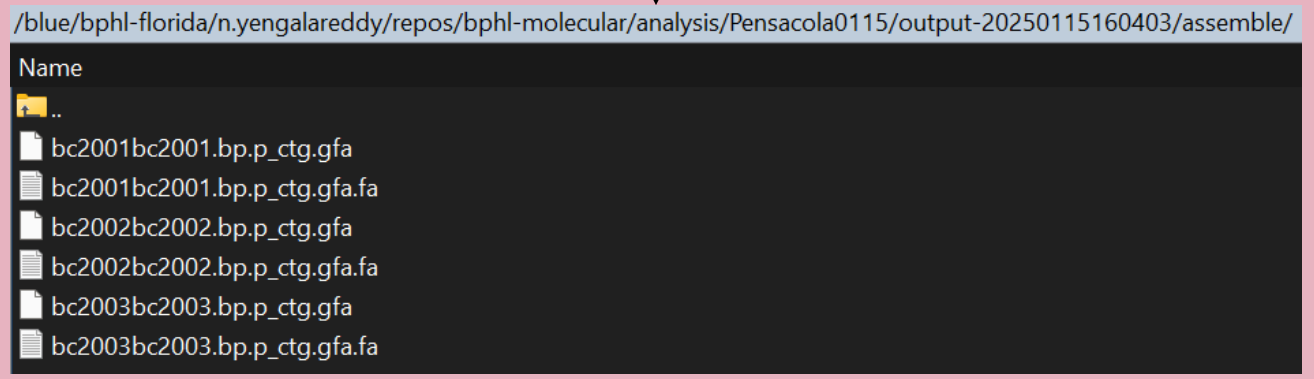
3. bc2002bc2002.variants_snpeff.ann.vcf:

ID,Drug,Gene,Mutation
B9J08_001448,FLC,ERG11,Y132F;K143R;Phe126Leu

B9J08_005576,FLC,,Cys110Phe

Application Cont.





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Application Cont.

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Pensacola0115/output-20250115160403/

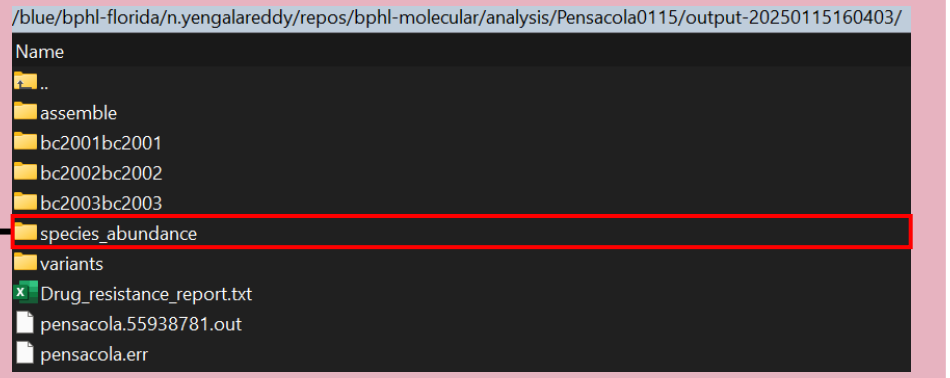
Name
- ..
- assemble
- bc2001bc2001
- bc2002bc2002
- bc2003bc2003
- species_abundance
- variants
- Drug_resistance_report.txt
- pensacola.55938781.out
- pensacola.err

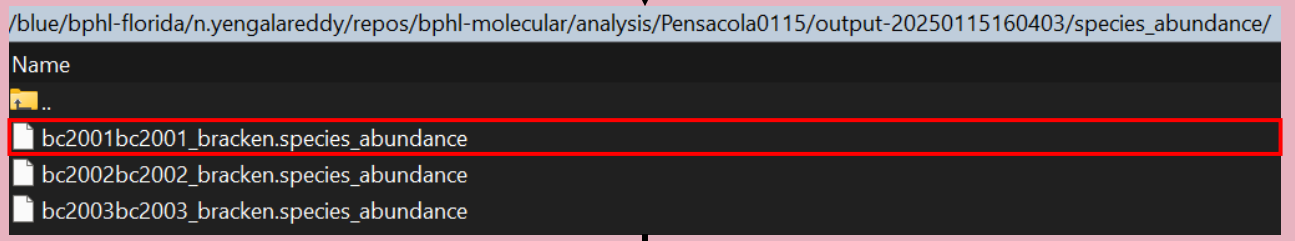
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Pensacola0115/output-20250115160403/bc2001bc2001/

Name
- ..
- assemble
- kraken_out
- qc
- bc2001bc2001.bam
- bc2001bc2001.bam.pbi
- bc2001bc2001.coverage.txt
- bc2001bc2001.fastq.gz
- bc2001bc2001.mapped.bam
- bc2001bc2001.mapped.bam.bai
- bc2001bc2001.mapped.bam.depth
- bc2001bc2001.mapped.bam.mpileup
- bc2001bc2001.variants_bcftools.vcf
- bc2001bc2001.variants_snpeff.ann.vcf
- bc2001bc2001_snpeff.genes.txt
- bc2001bc2001_snpeff.html

#rname	startpos	endpos	numreads	covbases	coverage	meandepth	meanbaseq	meanmapq
PEKT02000001_C_auris_B8441	1	1083522	9352	1083188	99.9692	69.597	86.1	60
PEKT02000002_C_auris_B8441	1	1280737	13641	1279564	99.9084	85.9919	85.8	53.4
PEKT02000003_C_auris_B8441	1	1047474	11412	1033718	98.6867	86.1431	86	60
PEKT02000004_C_auris_B8441	1	887381	9538	886816	99.9363	86.9957	85.9	60
PEKT02000005_C_auris_B8441	1	639401	7825	638481	99.8561	94.9978	86.1	59.8
PEKT02000006_C_auris_B8441	1	776876	10316	776387	99.9371	107.672	86.1	60
PEKT02000007_C_auris_B8441	1	3195935	28493	3191857	99.8724	71.366	86.1	59.9
PEKT02000008_C_auris_B8441	1	898131	10741	894257	99.5687	95.8412	85.7	59.6
PEKT02000009_C_auris_B8441	1	1007143	12759	998219	99.1139	102.062	86	59.7
PEKT02000010_C_auris_B8441	1	1402902	15384	1399198	99.736	88.2547	86	60
PEKT02000012_C_auris_B8441	1	65067	978	63688	97.8806	110.579	85.9	59.8
PEKT02000013_C_auris_B8441	1	38216	931	38159	99.8508	166.528	85.9	59.8
PEKT02000014_C_auris_B8441	1	11792	159	11765	99.771	73.7636	85.3	60
PEKT02000011_C_auris_B8441	1	20765	0	0	0	0	0	0
PEKT02000015_C_auris_B8441	1	10617	0	0	0	0	0	0

Application Cont.





name	taxonomy_id	taxonomy_lvl	kraken_assigned_reads	added_reads	new_est_reads	fraction_total_reads
[Candida] auris	498019	S	127942	68	128010	0.94999
Debaryomyces hansenii	4959	S	4961	15	4976	0.03693
Brettanomyces nanus	13502	S	40	1	41	0.0003
Brettanomyces bruxellensis	5007	S	1	0	1	0.00001
Aspergillus nidulans	162425	S	3	0	3	0.00002
Homo sapiens	9606	S	1681	9	1690	0.01254
Chryseobacterium aureum	2497456	S	13	7	20	0.00015
Ferrimonas sp. YFM	3028878	S	1	1	2	0.00001
Finegoldia magna	1260	S	1	2	3	0.00002

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Application Cont.

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Pensacola0115/output-20250115160403/

Name
- ..
- assemble
- bc2001bc2001
- bc2002bc2002
- bc2003bc2003
- species_abundance
- variants
- Drug_resistance_report.txt
- pensacola.55938781.out
- pensacola.err

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/Pensacola0115/output-20250115160403/variants/

Name
- ..
- bc2001bc2001.variants_bcftools.vcf
- bc2001bc2001.variants_snpeff.ann.vcf
- bc2001bc2001_snpeff.genes.txt
- bc2001bc2001_snpeff.html
- bc2002bc2002.variants_bcftools.vcf
- bc2002bc2002.variants_snpeff.ann.vcf
- bc2002bc2002_snpeff.genes.txt
- bc2002bc2002_snpeff.html
- bc2003bc2003.variants_bcftools.vcf
- bc2003bc2003.variants_snpeff.ann.vcf
- bc2003bc2003_snpeff.genes.txt
- bc2003bc2003_snpeff.html

The following table is formatted as tab separated values.

#GeneName	GeneId	TranscriptId	BioType	variants_impact_HIGH	variants_impact_LOW	variants_impact_MODERATE	variants_impact_MODIFIER	variants_effect_conservative_inframe_deletion	variants_effect_conservative_inframe_insertion	variants_effect_disruptive_inframe_deletion	variants_effect_disruptive_inframe_insertion	variants_effect_downstream_gene_variant	variants_effect_frameshift_variant	variants_effect_intron_variant
B9J08_000001	B9J08_000001	B9J08_000001-T	protein_coding	0	1	0	4	0	0	0	0	2	0	0
B9J08_000002	B9J08_000002	B9J08_000002-T	protein_coding	0	0	0	4	0	0	0	0	2	0	0
B9J08_000003	B9J08_000003	B9J08_000003-T	protein_coding	0	0	0	5	0	0	0	0	2	0	0
B9J08_000004	B9J08_000004	B9J08_000004-T	protein_coding	0	0	0	7	0	0	0	0	5	0	0
B9J08_000005	B9J08_000005	B9J08_000005-T	protein_coding	0	0	1	21	0	0	0	0	19	0	0
B9J08_000006	B9J08_000006	B9J08_000006-T	protein_coding	0	0	1	24	0	0	0	0	23	0	0
B9J08_000007	B9J08_000007	B9J08_000007-T	protein_coding	0	1	0	39	0	0	0	0	3	0	0
B9J08_000008	B9J08_000008	B9J08_000008-T	protein_coding	0	11	7	57	0	0	0	0	8	0	0
B9J08_000009	B9J08_000009	B9J08_000009-T	protein_coding	0	10	2	62	0	0	0	0	26	0	0
B9J08_000010	B9J08_000010	B9J08_000010-T	protein_coding	0	0	0	64	0	0	0	0	10	0	0
B9J08_000011	B9J08_000011	B9J08_000011-T	protein_coding	0	3	4	56	0	0	0	0	15	0	0
B9J08_000012	B9J08_000012	B9J08_000012-T	protein_coding	0	2	0	22	0	0	0	0	8	0	0
B9J08_000013	B9J08_000013	B9J08_000013-T	protein_coding	0	1	1	25	0	0	0	0	10	0	0
B9J08_000014	B9J08_000014	B9J08_000014-T	protein_coding	0	0	1	24	0	0	0	0	9	0	0
B9J08_000015	B9J08_000015	B9J08_000015-T	protein_coding	0	4	1	22	0	0	0	0	10	0	0
B9J08_000016	B9J08_000016	B9J08_000016-T	protein_coding	0	4	0	24	0	0	0	0	8	0	0
B9J08_000017	B9J08_000017	B9J08_000017-T	protein_coding	0	1	4	27	0	0	0	0	12	0	0
B9J08_000018	B9J08_000018	B9J08_000018-T	protein_coding	0	4	0	100	0	0	0	0	82	0	0
B9J08_000019	B9J08_000019	B9J08_000019-T	protein_coding	0	21	10	57	0	0	0	0	46	0	0
B9J08_000020	B9J08_000020	B9J08_000020-T	protein_coding	0	1	0	90	0	0	0	0	9	0	0
B9J08_000021	B9J08_000021	B9J08_000021-T	protein_coding	0	0	1	80	0	0	0	0	12	0	0
B9J08_000022	B9J08_000022	B9J08_000022-T	protein_coding	0	0	1	22	0	0	0	0	14	0	0
B9J08_000023	B9J08_000023	B9J08_000023-T	protein_coding	0	5	2	22	0	0	0	0	5	0	0
B9J08_000024	B9J08_000024	B9J08_000024-T	protein_coding	0	0	0	31	0	0	0	0	12	0	0
B9J08_000025	B9J08_000025	B9J08_000025-T	protein_coding	0	0	2	28	0	0	0	0	14	0	0
B9J08_000026	B9J08_000026	B9J08_000026-T	protein_coding	0	2	1	30	0	0	0	0	16	0	0

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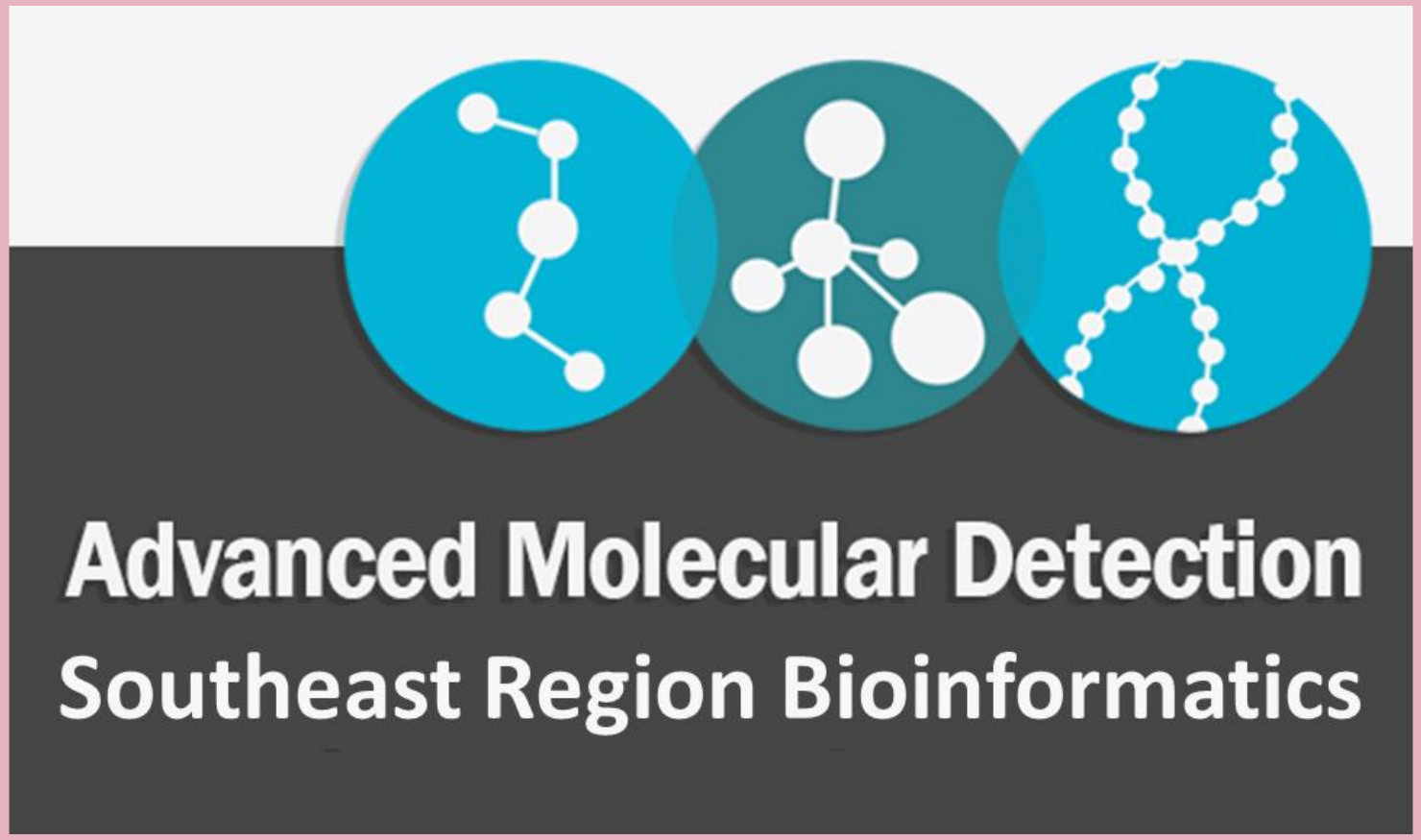
Conclusion

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



Questions?

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