

MycosNP

Advanced Molecular Detection
Southeast Region Bioinformatics

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Overview

Purpose:

This nextflow pipeline is developed for fungal whole-genome sequencing (e.g., *Candida auris*). This pipeline performs QC, read alignment to a masked reference, and SNP calling to generate phylogenetic outputs.

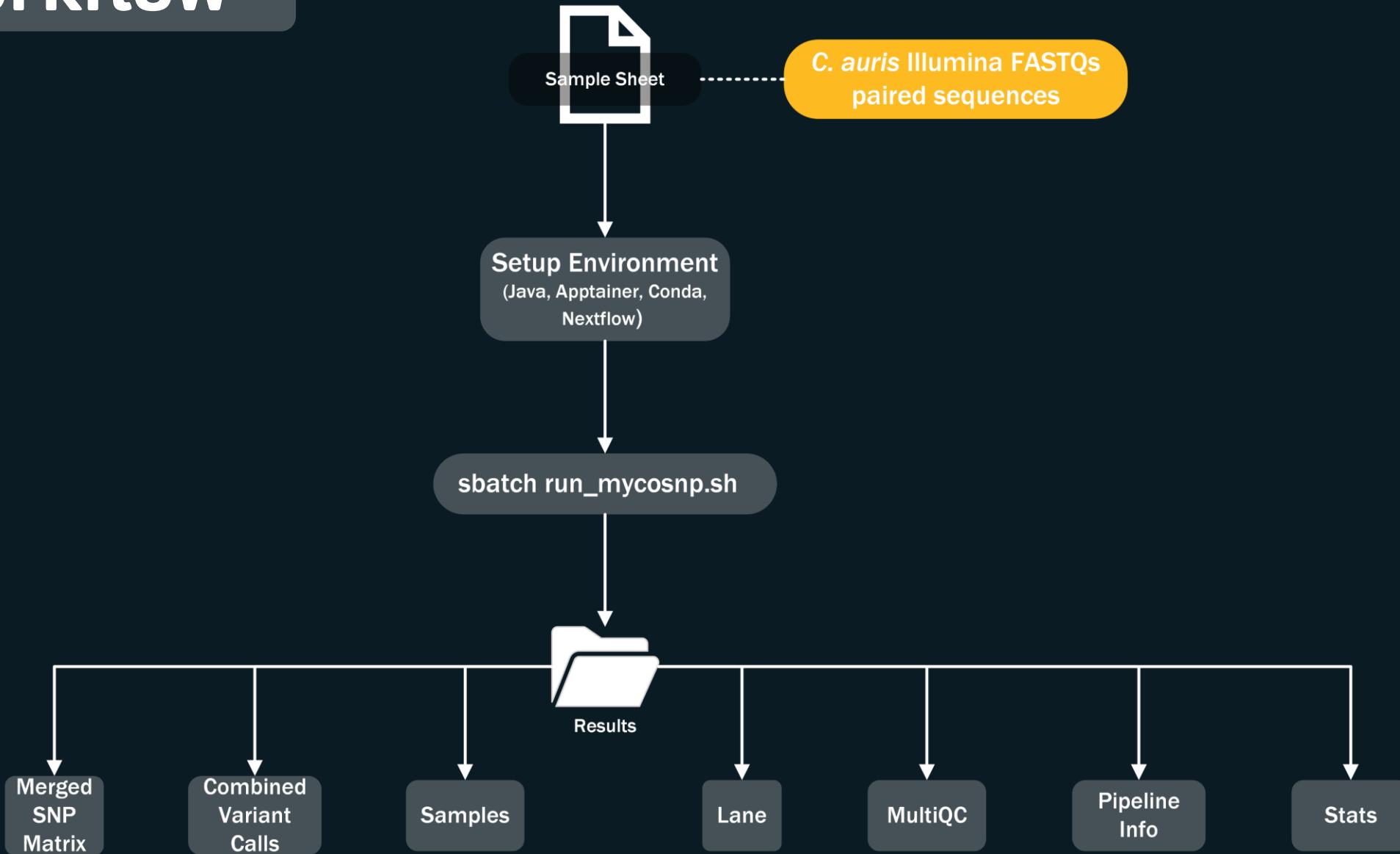
Usage:

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

Dependencies:

Nextflow
Apptainer
Java
Reference Genome

Workflow



Application

Objective

Use *C. auris* Illumina FASTQs paired sequences from NCBI database, and use Mycosnp to analyze the samples

Application Cont.

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/
Name
..  
assets  
bin  
conf  
docs  
input  
lib  
Misc  
modules  
mycosnp_results  
subworkflows  
work  
workflows  
CHANGELOG.md  
CITATIONS.md  
CODE_OF_CONDUCT.md  
code-of-conduct.md  
CONTRIBUTING.md  
DISCLAIMER.md  
LICENSE  
main.nf  
modules.json  
mycosnp.15654644.err  
mycosnp.15654644.out  
nextflow.config  
nextflow_schema.json  
open_practices.md  
README.md  
rules_of_behavior.md  
run_mycosnp.sh  
tower.yml
```

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/input/
Name
..  
samplesheet.csv  
SRR35732983_R1.fastq.gz  
SRR35732983_R2.fastq.gz  
SRR35732984_R1.fastq.gz  
SRR35732984_R2.fastq.gz  
SRR35732985_R1.fastq.gz  
SRR35732985_R2.fastq.gz  
SRR35732986_R1.fastq.gz  
SRR35732986_R2.fastq.gz  
SRR35732987_R1.fastq.gz  
SRR35732987_R2.fastq.gz  
SRR35732988_R1.fastq.gz  
SRR35732988_R2.fastq.gz  
SRR35732989_R1.fastq.gz  
SRR35732989_R2.fastq.gz  
SRR35732990_R1.fastq.gz  
SRR35732990_R2.fastq.gz  
SRR35732991_R1.fastq.gz  
SRR35732992_R1.fastq.gz  
SRR35732992_R2.fastq.gz  
SRR35732993_R1.fastq.gz  
SRR35732993_R2.fastq.gz  
SRR35732994_R1.fastq.gz  
SRR35732994_R2.fastq.gz  
SRR35732995_R1.fastq.gz  
SRR35732995_R2.fastq.gz
```

cd blue/bphl-<state>/<user>/repos/bphl-molecular/
git clone https://github.com/CDCgov/mycosnp-nf
mkdir analysis/
cd analysis/
cp /blue/bphl-<state>/<user>/repos/bphl-molecular/mycosnp/

Application Cont.

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

# Environment Setup
module load conda
conda activate Nextflow
module load apptainer

# Paths
WORKDIR="${PWD}"
SAMPLESHEET="${WORKDIR}/input/samplesheet.csv"
FASTA="/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/assets/precomputed/reference/masked/GCA_016772135.1_ASM1677213v1_genomic/masked/reference.fa"
OUTDIR="${WORKDIR}/mycosnp_results"
NF_WORK="${WORKDIR}/work"

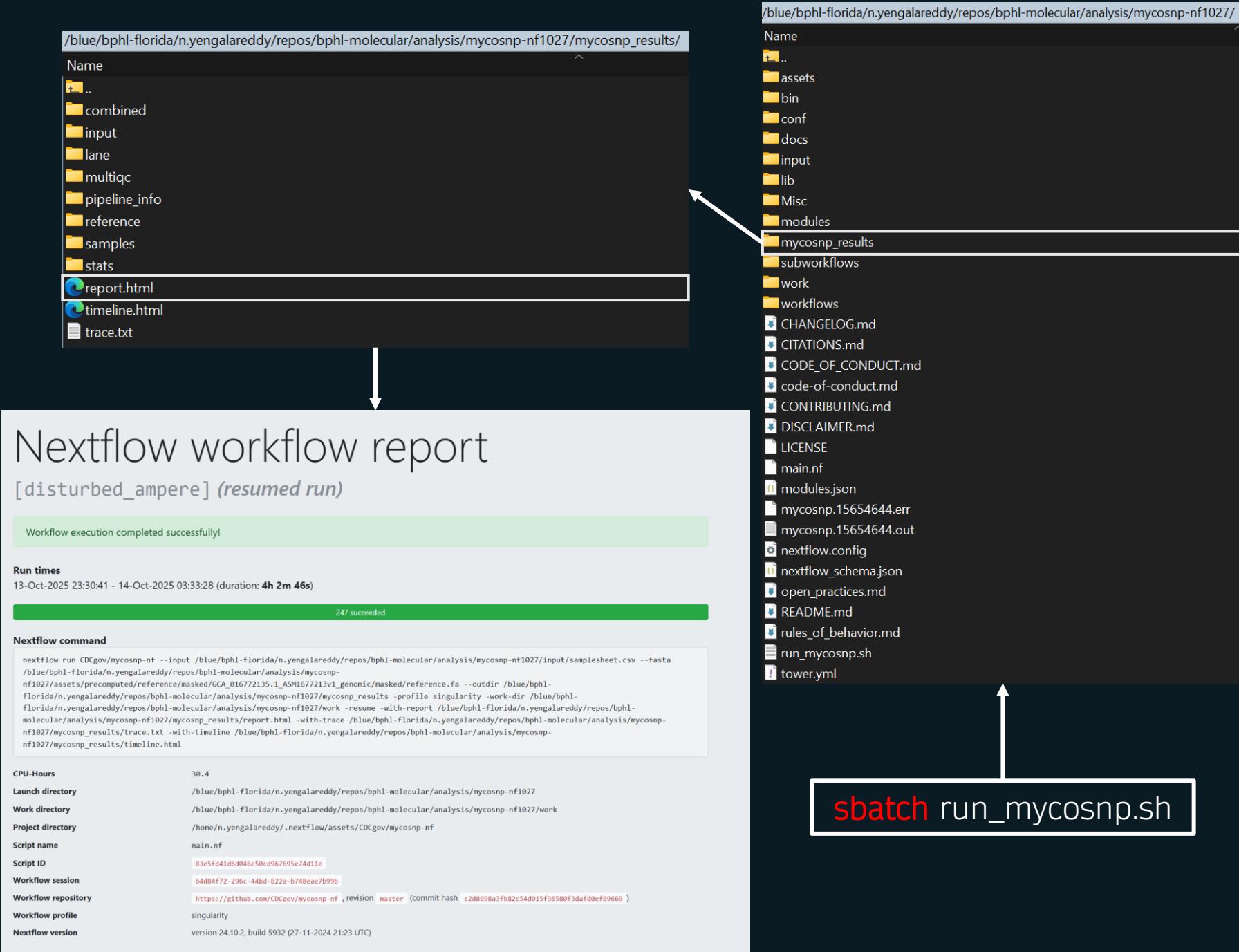
mkdir -p "${OUTDIR}" "${NF_WORK}"

# Run
nextflow run CDCgov/mycosnp-nf \
--input "${SAMPLESHEET}" \
--fasta "${FASTA}" \
--outdir "${OUTDIR}" \
-profile singularity \
-work-dir "${NF_WORK}" \
-resume \
-with-report "${OUTDIR}/report.html" \
-with-trace "${OUTDIR}/trace.txt" \
-with-timeline "${OUTDIR}/timeline.html"

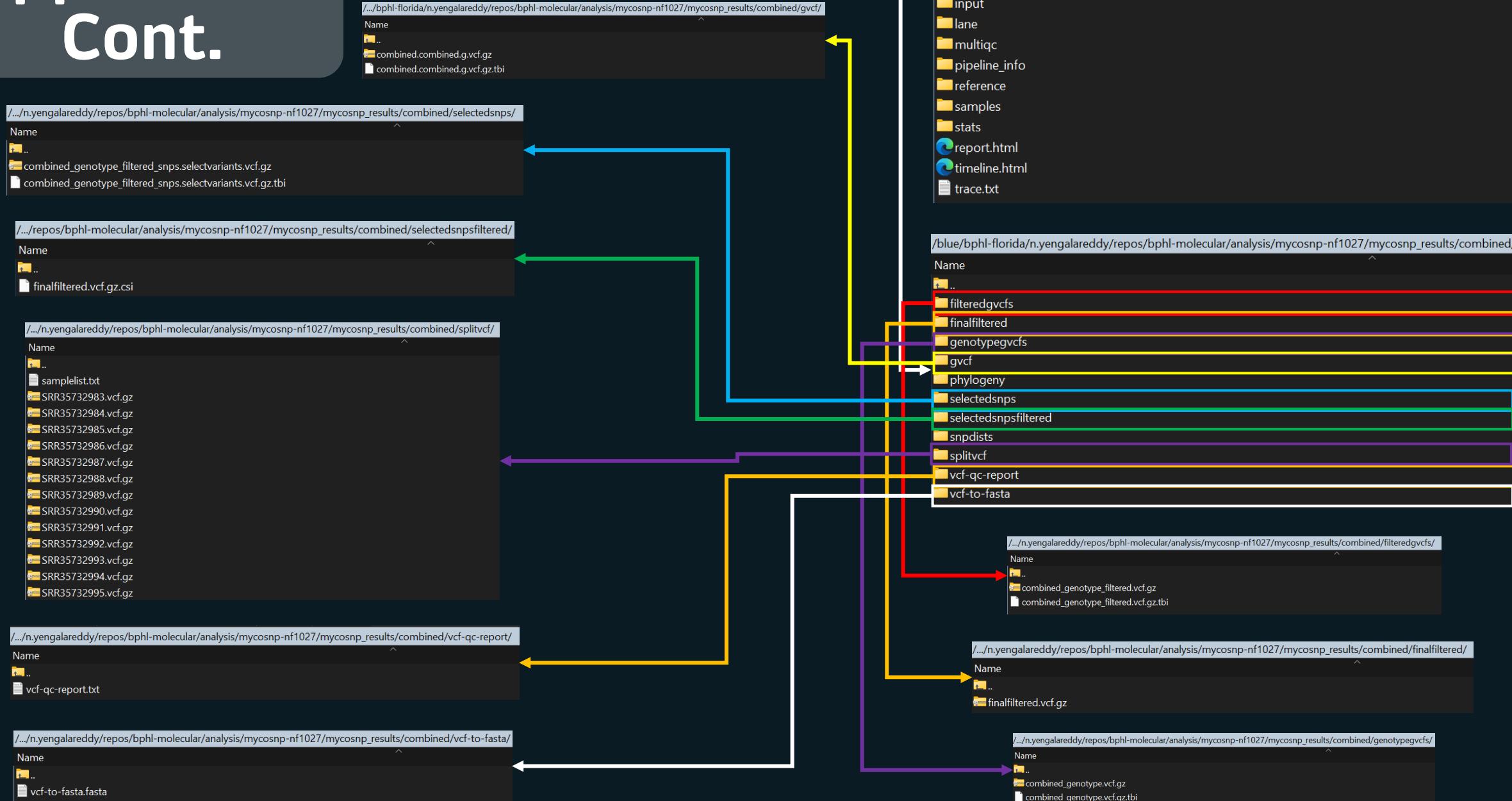
echo "[DONE] Results in ${OUTDIR}"
```

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/
Name
..
assets
bin
conf
docs
input
lib
Misc
modules
mycosnp_results
subworkflows
work
workflows
CHANGELOG.md
CITATIONS.md
CODE_OF_CONDUCT.md
code-of-conduct.md
CONTRIBUTING.md
DISCLAIMER.md
LICENSE
main.nf
modules.json
mycosnp.15654644.err
mycosnp.15654644.out
nextflow.config
nextflow_schema.json
open_practices.md
README.md
rules_of_behavior.md
run_mycosnp.sh
tower.yml

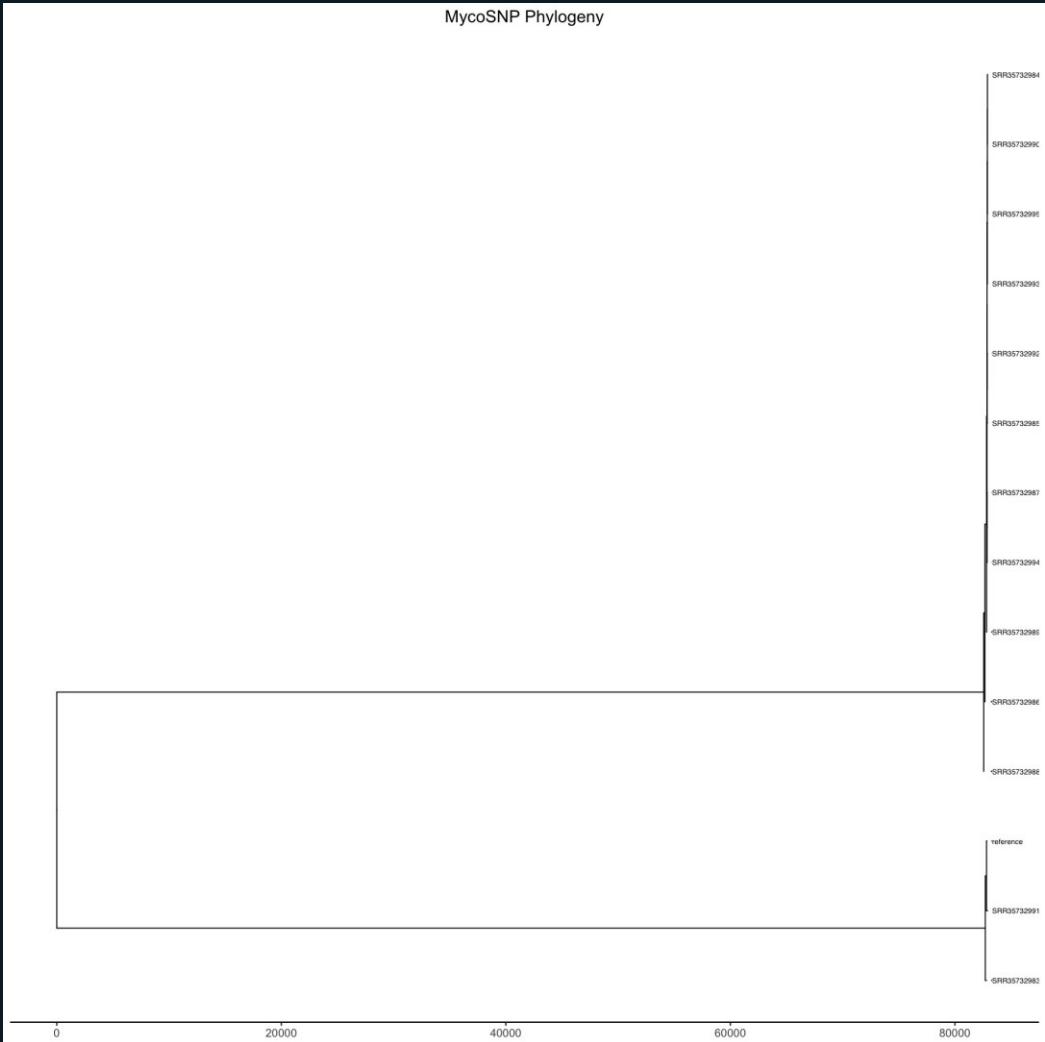
Application Cont.



Application Cont.

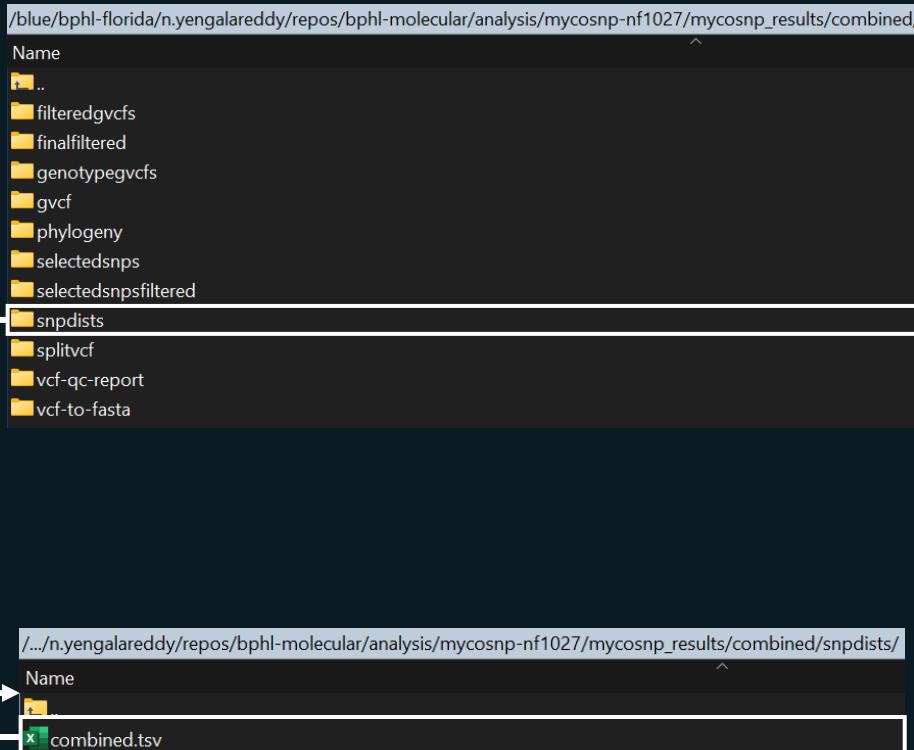


Application Cont.

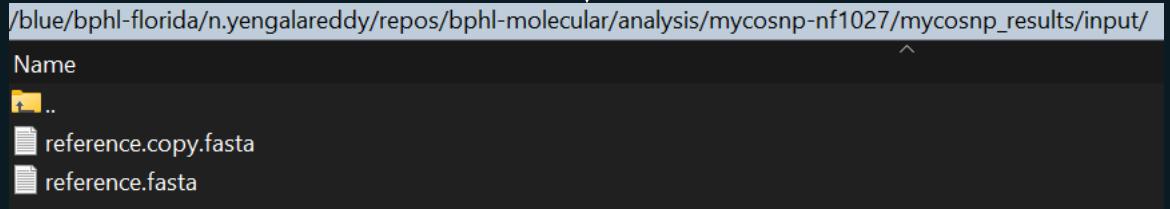
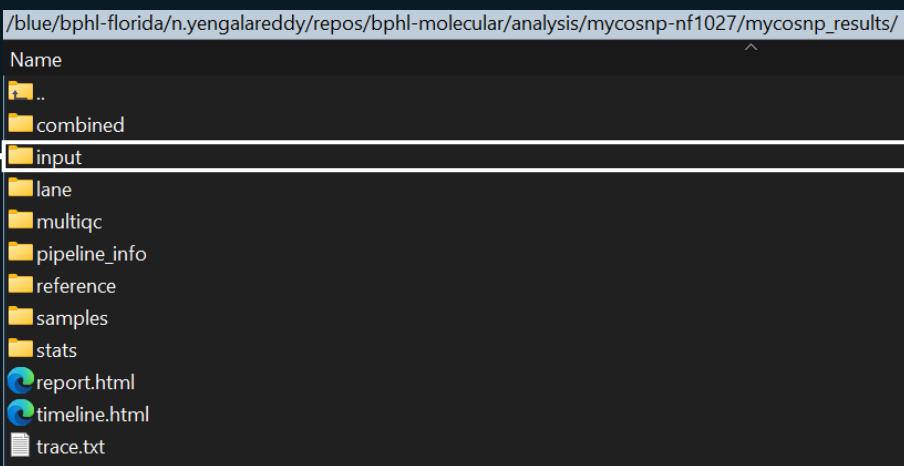


Application Cont.

reference	0	238	166464	166429	165527	166411	165416	166006	166488	124	166460	166144	166420	166480
SRR35732983	238	0	166428	166394	165493	166374	165381	165972	166453	316	166425	166109	166385	166445
SRR35732984	166464	166428	0	73	74	63	120	67	21	166522	61	46	77	36
SRR35732985	166429	166394	73	0	60	51	102	55	77	166487	48	83	60	80
SRR35732986	165527	165493	74	60	0	53	108	56	79	165585	46	85	62	79
SRR35732987	166411	166374	63	51	53	0	97	12	63	166467	38	64	18	65
SRR35732988	165416	165381	120	102	108	97	0	96	124	165475	90	126	104	122
SRR35732989	166006	165972	67	55	56	12	96	0	66	166065	38	67	8	64
SRR35732990	166488	166453	21	77	79	63	124	66	0	166546	57	48	73	33
SRR35732991	124	316	166522	166487	165585	166467	165475	166065	166546	0	166518	166202	166478	166538
SRR35732992	166460	166425	61	48	46	38	90	38	57	166518	0	69	40	51
SRR35732993	166144	166109	46	83	85	64	126	67	48	166202	69	0	80	46
SRR35732994	166420	166385	77	60	62	18	104	8	73	166478	40	80	0	73
SRR35732995	166480	166445	36	80	79	65	122	64	33	166538	51	46	73	0



Application Cont.

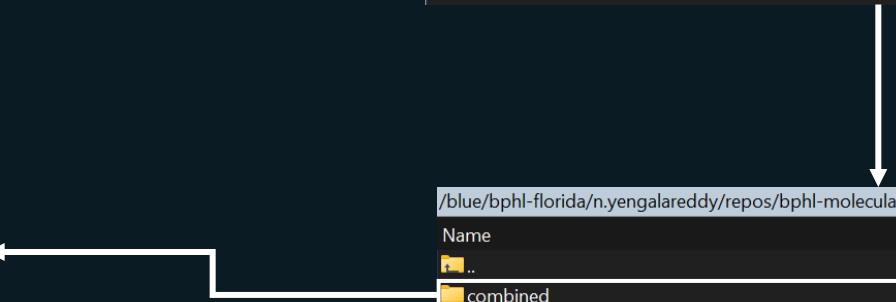


Application Cont.

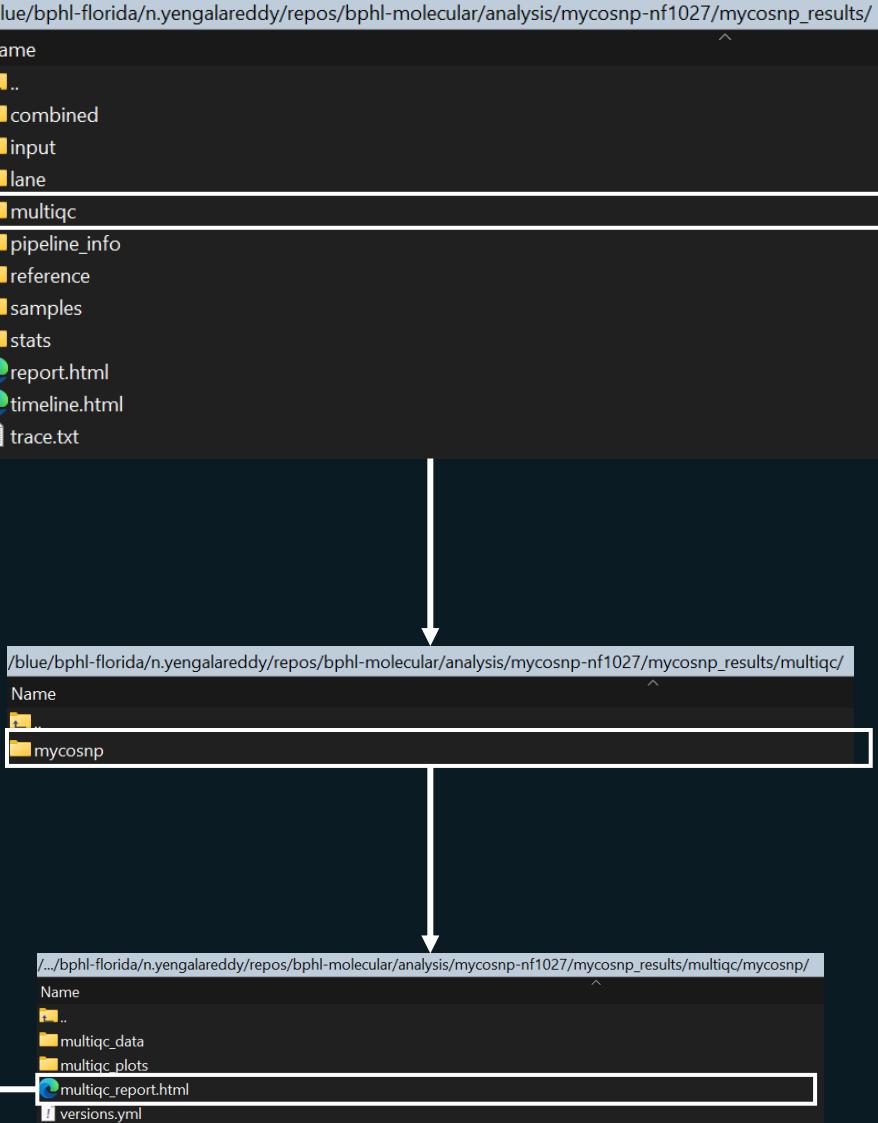
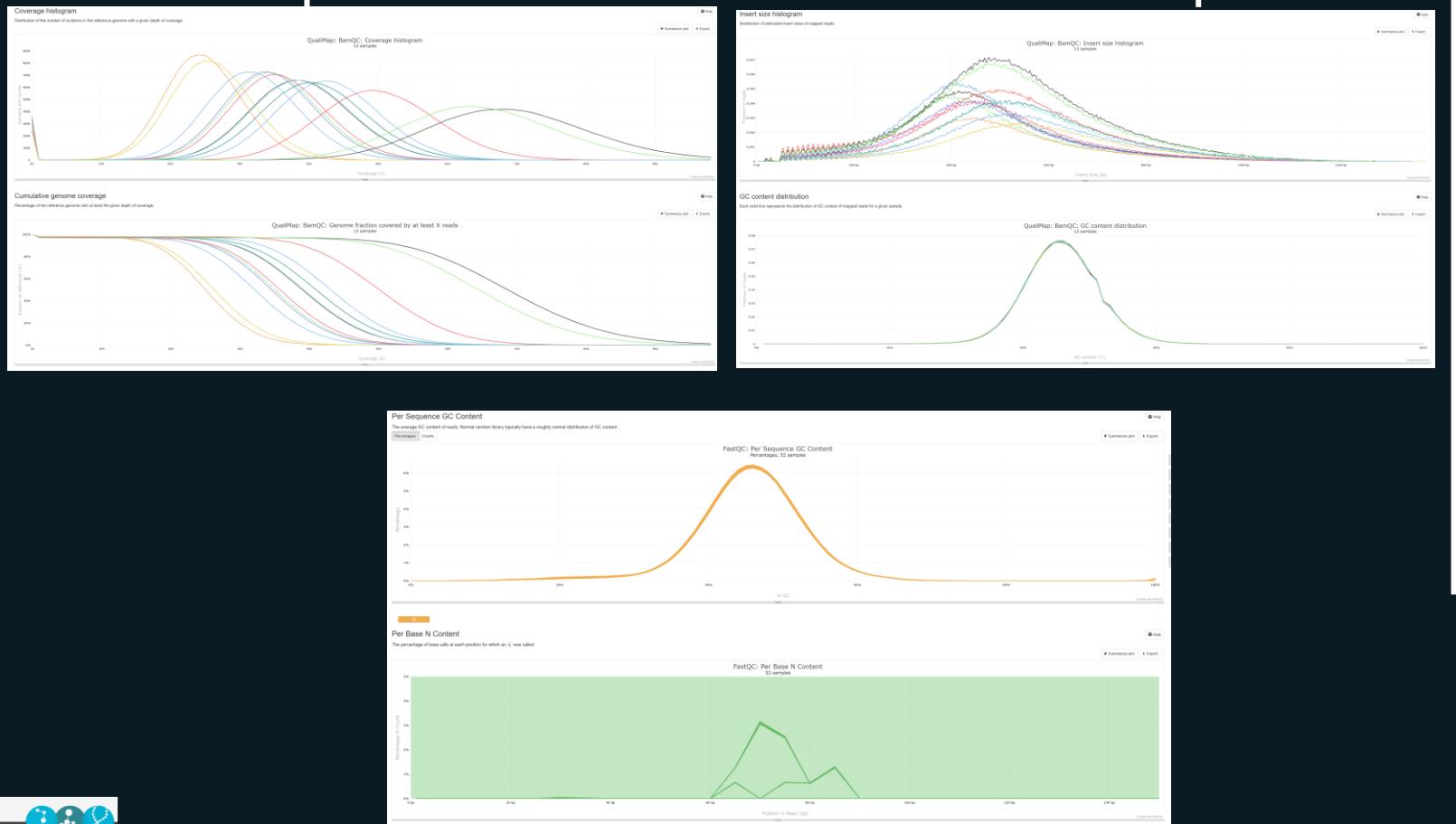
```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/lane/combined/
Name
..
SRR35732983_R1.fastq.gz
SRR35732983_R2.fastq.gz
SRR35732984_R1.fastq.gz
SRR35732984_R2.fastq.gz
SRR35732985_R1.fastq.gz
SRR35732985_R2.fastq.gz
SRR35732986_R1.fastq.gz
SRR35732986_R2.fastq.gz
SRR35732987_R1.fastq.gz
SRR35732987_R2.fastq.gz
SRR35732988_R1.fastq.gz
SRR35732988_R2.fastq.gz
SRR35732989_R1.fastq.gz
SRR35732989_R2.fastq.gz
SRR35732990_R1.fastq.gz
SRR35732990_R2.fastq.gz
SRR35732991_R1.fastq.gz
SRR35732991_R2.fastq.gz
SRR35732992_R1.fastq.gz
SRR35732992_R2.fastq.gz
SRR35732993_R1.fastq.gz
SRR35732993_R2.fastq.gz
SRR35732994_R1.fastq.gz
SRR35732994_R2.fastq.gz
SRR35732995_R1.fastq.gz
SRR35732995_R2.fastq.gz
```

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/
Name
..
combined
input
lane
multiqc
pipeline_info
reference
samples
stats
report.html
timeline.html
trace.txt
```

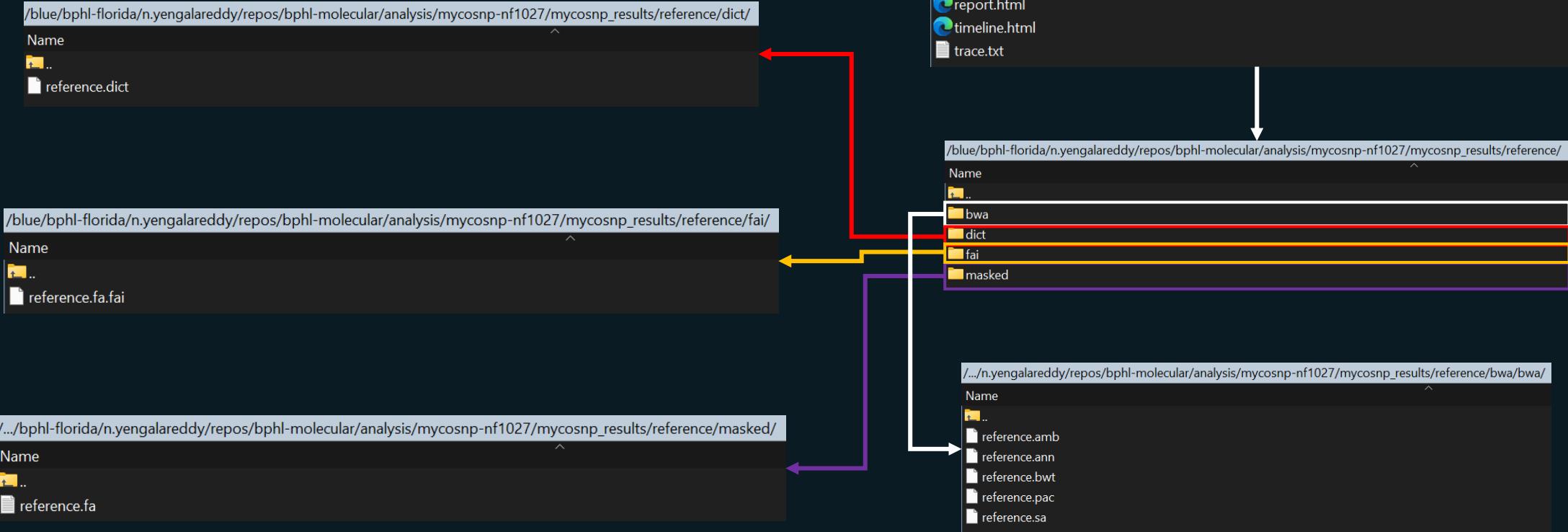
```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/lane/
Name
..
combined
```



Application Cont.



Application Cont.



Application Cont.

```
./.../n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/samples/SRR35732983/
```

Name

- ..
- faqcis
- fastqc_post
- fastqc_raw
- finalbam
- qc_report
- variant_calling

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/
```

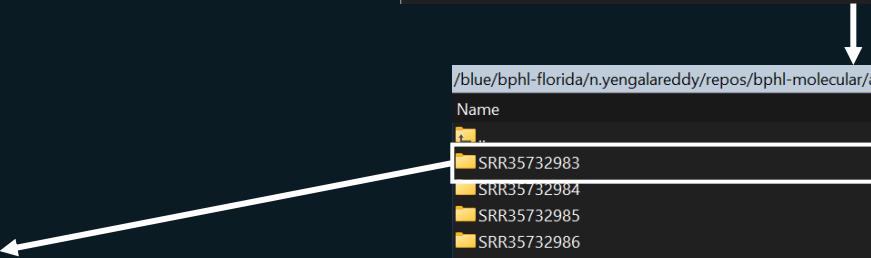
Name

- ..
- combined
- input
- lane
- multiqc
- pipeline_info
- reference
- samples**
- stats
- report.html
- timeline.html
- trace.txt

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/samples/
```

Name

- ..
- SRR35732983**
- SRR35732984
- SRR35732985
- SRR35732986
- SRR35732987
- SRR35732988
- SRR35732989
- SRR35732990
- SRR35732991
- SRR35732992
- SRR35732993
- SRR35732994
- SRR35732995



Application Cont.

```
./n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/stats/samtools_flagstat/
Name
..
SRR35732983.flagstat
SRR35732984.flagstat
SRR35732985.flagstat
SRR35732986.flagstat
SRR35732987.flagstat
SRR35732988.flagstat
SRR35732989.flagstat
SRR35732990.flagstat
SRR35732991.flagstat
SRR35732992.flagstat
SRR35732993.flagstat
SRR35732994.flagstat
SRR35732995.flagstat
```

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/
Name
..
combined
input
lane
multiqc
pipeline_info
reference
samples
stats
report.html
timeline.html
trace.txt
```

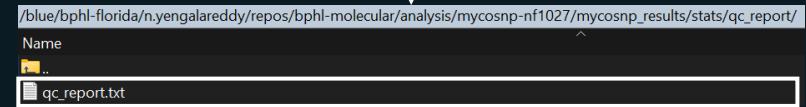
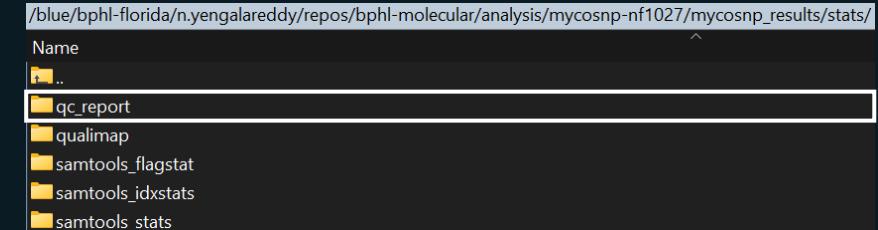
```
./n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/stats/samtools_idxstats/
Name
..
SRR35732983.idxstats
SRR35732984.idxstats
SRR35732985.idxstats
SRR35732986.idxstats
SRR35732987.idxstats
SRR35732988.idxstats
SRR35732989.idxstats
SRR35732990.idxstats
SRR35732991.idxstats
SRR35732992.idxstats
SRR35732993.idxstats
SRR35732994.idxstats
SRR35732995.idxstats
```

```
/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/stats/
Name
..
qc_report
qualimap
samtools.flagstat
samtools_idxstats
samtools_stats
```

This file was produced by samtools stats (1.15+htslib-1.15) and can be plotted using plot-bamstats
This file contains statistics for all reads.
The command line was: stats --threads 1 --reference reference.fa SRR35732983.bam
CHK, Checksum [2]Read Names [3]Sequences [4]Qualities
CKH, CRC32 of reads which passed filtering followed by addition (32bit overflow)
CHK 1ab9b139 a55f9660 dd437f18
Summary Numbers. Use grep "SN" | cut -f 2- to extract this part.
SN total sequences: 3809318 # excluding supplementary and secondary reads
SN filtered sequences: 0
SN is sorted: 1
SN sequences: 3809318
SN ist fragments: 190381
SN last fragments: 1986287
SN reads mapped: 3521326 # paired-end technology bit set + both mates mapped
SN reads unmapped: 281046
SN reads paired: 3503676 # proper-pair bit set
SN reads paired: 3809318 # paired-end technology bit set
SN reads duplicated: 0 # PCR or optical duplicate bit set
SN reads MQ: 3077 # mapped and MQ=0
SN reads aligned: 0
SN non-primary alignments: 0
SN supplementary alignments: 12882
SN total length: 569049190 # ignores clipping
SN total first fragment length: 284286648 # ignores clipping
SN total last fragment length: 284762542 # ignores clipping
SN bases mapped: 527083664 # ignores clipping
SN bases mapped (cigar): 522874252 # more accurate
SN bases aligned: 0
SN bases duplicated: 0
SN mismatches: 3210487 # from NM fields
SN error rate: 6.140075e-03 # mismatches / bases mapped (cigar)
SN average length: 149
SN average last fragment length: 149
SN maximum last fragment length: 151
SN maximum length: 151
SN maximum first fragment length: 151
SN maximum last fragment length: 151
SN average quality: 32.2
SN insert size average: 447.1
SN insert size standard deviation: 161.6
SN inward oriented pairs: 1733116
SN outward oriented pairs: 25224
SN pairs with other orientation: 719
SN pairs on different chromosomes: 3504
SN percentage of properly paired reads (%): 92.0

Application Cont.

Sample Name	Reads Before Trimming	GC Before Trimming	Average Q Score Before Trimming	Reference Length Coverage Before Trimming	Reads After Trimming	Paired Reads After Trimming	Unpaired Reads After Trimming	GC After Trimming	Average Q Score After Trimming	Reference Length Coverage After Trimming	Mean Coverage Depth	Reads Mapped	Genome Fraction at 10X
SRR35732983	4778776	44.68%	31.53	57.41 4685273 (98.04 %)	4607102 (98.33 %)	78171 (1.67 %)	44.68%	31.56	56.41	42.15 3528272 (92.62%)	97.61%		
SRR35732984	4268062	44.85%	31.44	51.35 4186243 (98.08 %)	4116364 (98.33 %)	69879 (1.67 %)	44.83%	31.47	50.46	38.08 3202595 (93.29%)	97.34%		
SRR35732985	3811464	44.86%	31.43	45.92 3739364 (98.11 %)	3675820 (98.30 %)	63544 (1.70 %)	44.84%	31.46	45.12	34.13 2869029 (93.42%)	97.33%		
SRR35732986	2682508	44.84%	31.35	32.38 2631790 (98.11 %)	2585454 (98.24 %)	46336 (1.76 %)	44.81%	31.38	31.8	24.32 2041642 (93.15%)	97.12%		
SRR35732987	3774968	44.76%	31.47	45.34 3699867 (98.01 %)	3637598 (98.32 %)	62269 (1.68 %)	44.75%	31.5	44.55	33.66 2832649 (93.23%)	97.29%		
SRR35732988	3851766	44.91%	31.39	46.31 3776454 (98.04 %)	3713004 (98.32 %)	63450 (1.68 %)	44.89%	31.41	45.51	34.75 2923616 (93.67%)	96.93%		
SRR35732989	2675970	44.87%	31.35	32.34 2668864 (99.73 %)	2664722 (99.84 %)	4142 (0.16 %)	44.79%	31.35	32.28	25.56 2142557 (93.02%)	97.17%		
SRR35732990	4212104	44.89%	31.44	50.81 4199411 (99.70 %)	4193910 (99.87 %)	5501 (0.13 %)	44.83%	31.45	50.72	39.96 3351841 (93.2%)	97.32%		
SRR35732991	5215094	44.66%	31.56	62.28 5183399 (99.39 %)	5177730 (99.89 %)	5669 (0.11 %)	44.60%	31.56	62.13	49.12 4132507 (92.15%)	97.98%		
SRR35732992	4017390	44.89%	31.4	48.49 4005727 (99.71 %)	4000514 (99.87 %)	5213 (0.13 %)	44.83%	31.4	48.4	38.26 3208558 (93.36%)	97.34%		
SRR35732993	3236024	44.97%	31.27	39.1 3227251 (99.73 %)	3222376 (99.85 %)	4875 (0.15 %)	44.89%	31.28	39.03	31.25 2619957 (93.83%)	97.15%		
SRR35732994	7225246	44.79%	31.46	86.87 7195576 (99.59 %)	7186828 (99.88 %)	8748 (0.12 %)	44.72%	31.47	86.7	67.3 5659495 (92.45%)	97.36%		
SRR35732995	6815548	44.75%	31.57	81.94 6788658 (99.61 %)	6781714 (99.90 %)	6944 (0.10 %)	44.70%	31.57	81.78	63.2 5310384 (92.37%)	97.35%		



Application Cont.

Qualimap Report: BAM QC

Qualimap Report: BAM QC

Qualimap

CONTENTS

- Input data & parameters
- Summary
- Coverage across reference
- Coverage Histogram
- Coverage Histogram (0-50X)
- Genome Fraction Coverage
- Duplication Rate Histogram
- Mapped Reads Nucleotide Content
- Mapped Reads GC-content Distribution
- Mapped Reads Clipping Profile
- Homopolymer Indels
- Mapping Quality Across Reference
- Mapping Quality Histogram
- Insert Size Across Reference
- Insert Size Histogram

Input data and parameters

Qualimap command line
qualimap bamqc -bam SRR35732983.bam -nw 400 -hm 3

Alignment

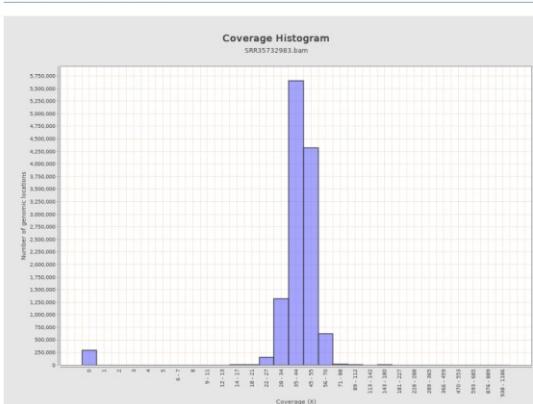
Command line:	bwa mem -t 4 /bwa/reference SRR35732983_1.trimmed.fastq.gz SRR35732983_2.trimmed.fastq.gz
Draw chromosome limits:	no
Analyze overlapping paired-end reads:	yes
Program:	bwa (0.7.17-r1188)
Analysis date:	Tue Oct 14 04:24:05 GMT 2025
Size of a homopolymer:	3
Skip duplicate alignments:	no
Number of windows:	400
BAM file:	SRR35732983.bam

Summary

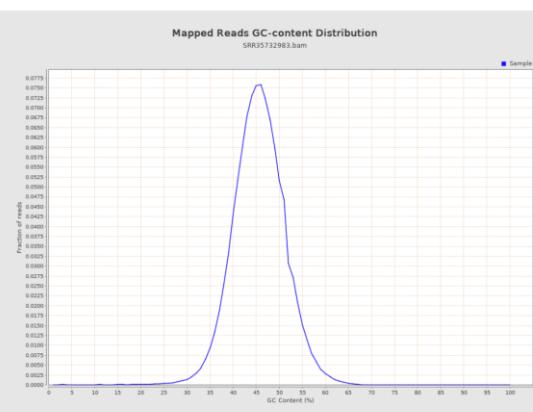
Globals

Reference size	12,406,106
Number of reads	3,809,318
Mapped reads	3,528,272 / 92.62%
Unmapped reads	281,046 / 7.38%
Mapped paired reads	3,528,272 / 92.62%
Mapped reads, first in pair	1,765,117 / 46.34%
Mapped reads, second in pair	1,763,155 / 46.29%
Mapped reads, both in pair	3,521,326 / 92.44%
Mapped reads, singletons	6,946 / 0.18%
Secondary alignments	0
Supplementary alignments	12,802 / 0.34%
Read min/max/mean length	30 / 151 / 149.53
Overlapping read pairs	270,783 / 14.22%
Duplicated reads (estimated)	1,220,151 / 32.03%
Duplication rate	31.43%
Clipped reads	138,274 / 3.63%

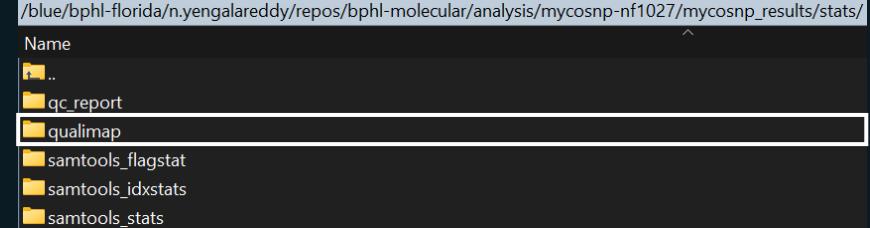
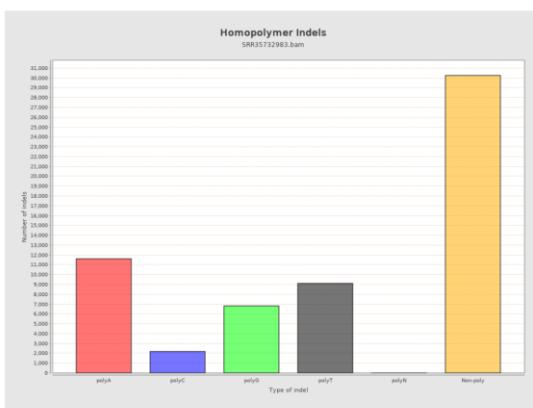
Coverage Histogram



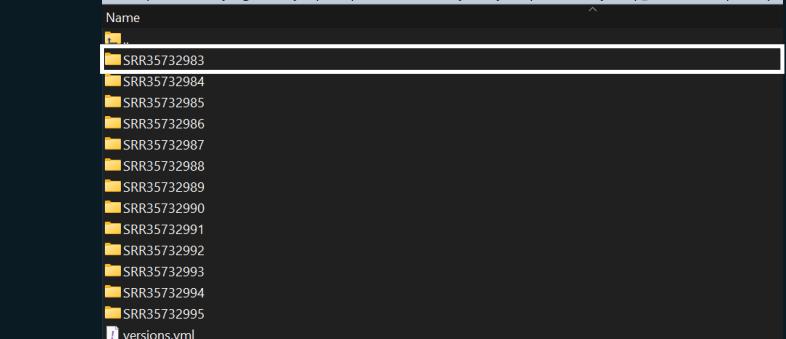
Mapped Reads GC-content Distribution



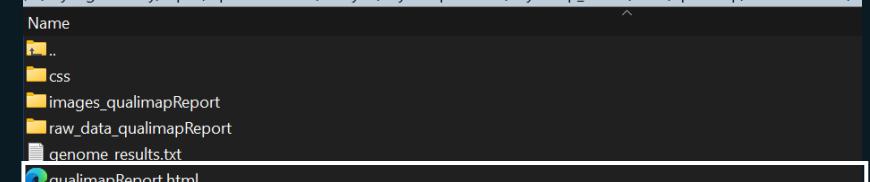
Homopolymer Indels



/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/stats/qualimap/



/.../n.yengalareddy/repos/bphl-molecular/analysis/mycosnp-nf1027/mycosnp_results/stats/qualimap/SRR35732983/



Conclusion



Fundamentals of
MycoSNP



Installation and
setup of MycoSNP in
HPG



Successfully executed
job query for
MycoSNP



Generated output
files



Advanced Molecular Detection

Southeast Region Bioinformatics

Questions?

bphl-sebioinformatics@flhealth.gov

Molly Mitchell, PhD
Bioinformatics Supervisor
Molly.Mitchell@flhealth.gov

Nikhil Reddy, MS
Bioinformatician
Nikhil.Yengala@flhealth.gov

Sam Bernhoft, MPH
Bioinformatician
Samantha.bernhoft@flhealth.gov