15 Dec 2025

BigBacter

Advanced Molecular Detection

Southeast Region Bioinformatics

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Overview

Purpose:

- A bacterial-genomic surveillance pipeline, this workflow builds and maintains population-level reference databases, performs accessory-genome clustering and core-genome SNV analysis, and generates phylogenies, distance matrices, and relatedness data to assess genetic relationships among isolates.

Usage:

- It is used by public-health and molecular-epidemiology teams to add new bacterial isolates, assign them to clusters, build per-isolate and combined phylogenetic trees, compute SNV and genome-distance matrices, and create QC plus summary reports — for outbreak detection, surveillance, and long-term genomic monitoring.

Dependencies:

- Nextflow + Apptainer/Singularity

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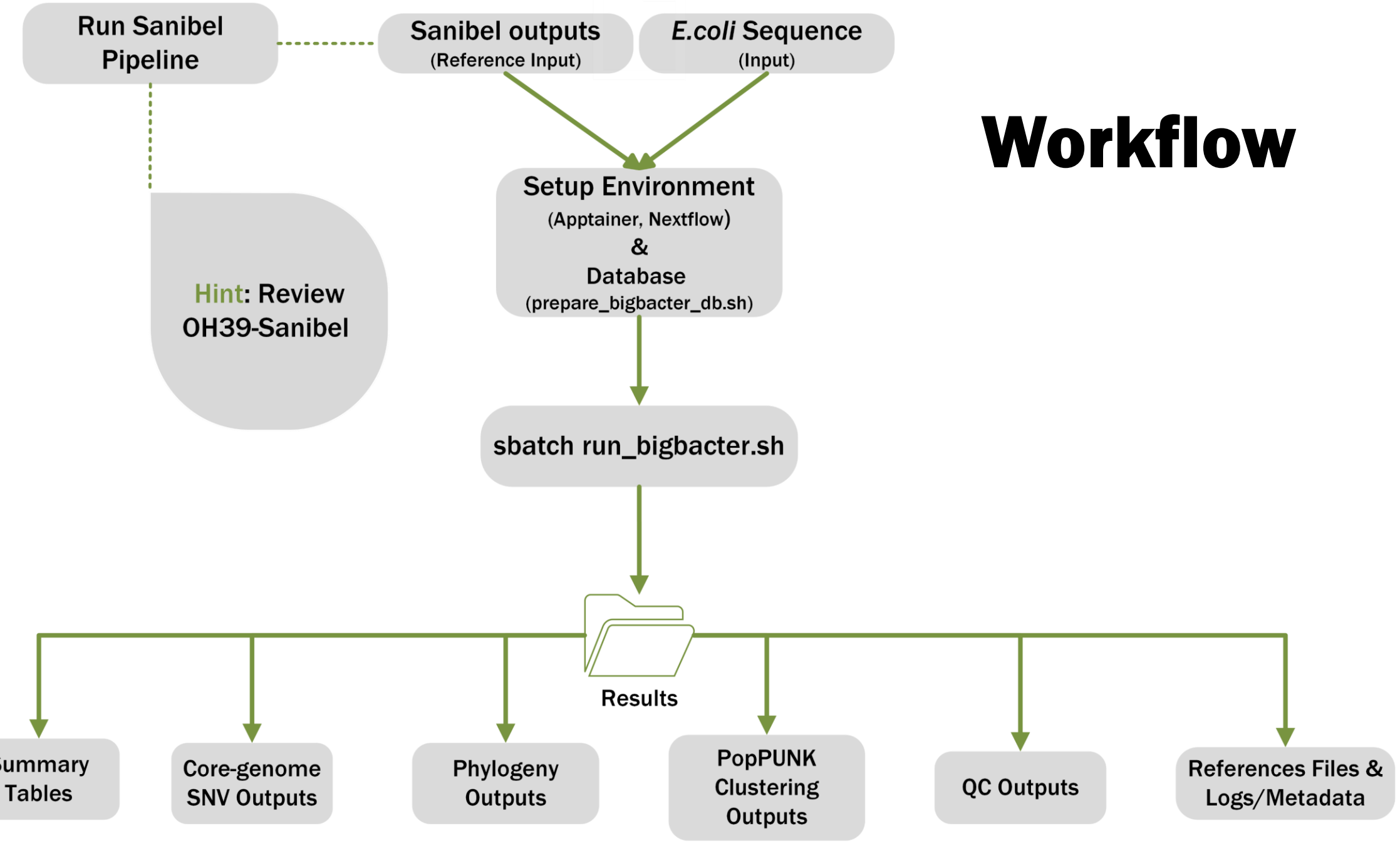
Workflow



Application

Objective

Use *E.coli* sequences and Sanibel output data with BigBacter to generate SNVs, PopPunk Clustering, and Phylogeny outputs .

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Application Cont.

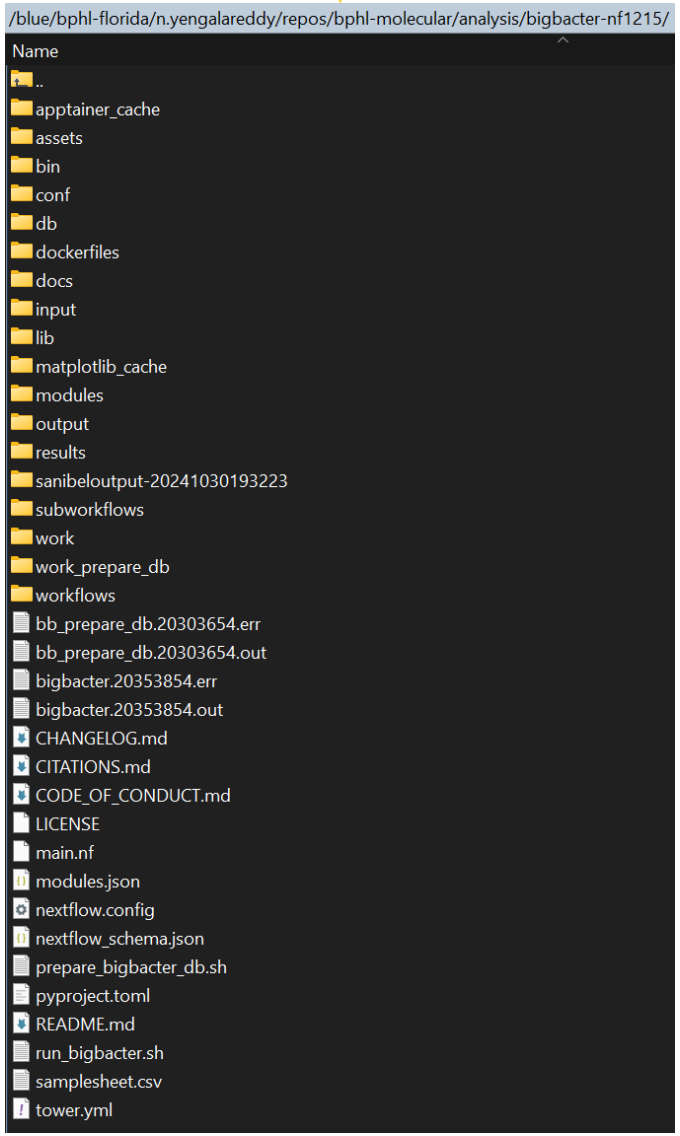
```
cd /blue/bphl-<state>/<user>/repos/bphl-molecular/

git clone https://github.com/DOH-JDJ0303/bigbacter-nf

mkdir analysis/

cd analysis/

cp /blue/bphl-<state>/<user>/repos/bphl-molecular/bigbacter-nf/
```



Application Cont.

```
 GNU nano 5.6.1                                  prepare_bigbacter_db.sh
#!/bin/bash
#SBATCH --account=bphl-umbrella
#SBATCH --qos=bphl-umbrella
#SBATCH --job-name=bb_prepare_db_ecoli
#SBATCH --mail-type=END,FAIL
#SBATCH --mail-user=nikhil.yengala@flhealth.gov
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=4
#SBATCH --mem=16G
#SBATCH --time=24:00:00
#SBATCH --output=bb_prepare_db.%j.out
#SBATCH --error=bb_prepare_db.%j.err

module purge
module load nextflow
module load apptainer

BASE=/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208
DBDIR="$BASE/db"

export NXF_WORK="$BASE/work_prepare_db"
export NXF_SINGULARITY_CACHEDIR="$BASE/apptainer_cache"
export MPLCONFIGDIR="$BASE/matplotlib_cache"
export NXF_SINGULARITY_HOME_MOUNT=true

mkdir -p "$DBDIR" "$NXF_WORK" "$NXF_SINGULARITY_CACHEDIR" "$MPLCONFIGDIR"

cd "$BASE"

nextflow run DOH-JDJ0303/bigbacter-nf \
  -r main \
  -profile singularity,escherichia_coli_db \
  -entry PREPARE_DB \
  --db "$DBDIR" \
  --max_cpus 4 \
  --max_memory 16.GB
```

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1215/

Name

- ..
- apptainer_cache
- assets
- bin
- conf
- db
- dockerfiles
- docs
- input
- lib
- matplotlib_cache
- modules
- output
- results
- sanibeloutput-20241030193223
- subworkflows
- work
- work_prepare_db
- workflows
- bb_prepare_db.20303654.err
- bb_prepare_db.20303654.out
- bigbacter.20353854.err
- bigbacter.20353854.out
- CHANGELOG.md
- CITATIONS.md
- CODE_OF_CONDUCT.md
- LICENSE
- main.nf
- modules.json
- nextflow.config
- nextflow_schema.json
- prepare_bigbacter_db.sh
- pyproject.toml
- README.md
- run_bigbacter.sh
- samplesheet.csv
- tower.yml

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Application Cont.

GNU nano 5.6.1 samplesheet.csv

```
sample,taxa,assembly,fastq_1,fastq_2
J4,Escherichia_coli,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208/sanibeloutput-20241030193223/J4/J4_assembly/J4.fasta,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf120
J7,Escherichia_coli,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208/sanibeloutput-20241030193223/J7/J7_assembly/J7.fasta,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf120
J8,Escherichia_coli,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208/sanibeloutput-20241030193223/J8/J8_assembly/J8.fasta,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf120
J9,Escherichia_coli,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208/sanibeloutput-20241030193223/J9/J9_assembly/J9.fasta,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf120
J17,Escherichia_coli,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208/sanibeloutput-20241030193223/J17/J17_assembly/J17.fasta,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-n
J52,Escherichia_coli,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208/sanibeloutput-20241030193223/J52/J52_assembly/J52.fasta,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-n
J100,Escherichia_coli,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208/sanibeloutput-20241030193223/J100/J100_assembly/J100.fasta,/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbact
```

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1215/db/Escherichia_coli/pp_db/

Name
..
0000000000.tar.gz

GNU nano 5.6.1 run_bigbacter.sh

```
#!/bin/bash
#SBATCH --account=bphl-umbrella
#SBATCH --qos=bphl-umbrella
#SBATCH --job-name=bigbacter_ecoli
#SBATCH --mail-type=END,FAIL
#SBATCH --mail-user=nikhil.yengala@flhealth.gov
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=24
#SBATCH --mem=128G
#SBATCH --time=48:00:00
#SBATCH --output=bigbacter.%j.out
#SBATCH --error=bigbacter.%j.err

module purge
module load nextflow
module load apptainer

BASE=/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1208
SAMPLESHEET="$BASE/samplesheet.csv"
OUTDIR="$BASE/output"
DBDIR="$BASE/db"

export NXF_WORK="$BASE/work"
export NXF_SINGULARITY_CACHEDIR="$BASE/apptainer_cache"
export MPLCONFIGDIR="$BASE/matplotlib_cache"
export NXF_SINGULARITY_HOME_MOUNT=true

mkdir -p "$OUTDIR" "$NXF_WORK" "$NXF_SINGULARITY_CACHEDIR" "$MPLCONFIGDIR"

cd "$BASE"

nextflow run DOH-JDJ0303/bigbacter-nf \
  -r main \
  -profile singularity \
  --input "$SAMPLESHEET" \
  --outdir "$OUTDIR" \
  --db "$DBDIR" \
  --threads "$SLURM_CPUS_PER_TASK" \
  --max_cpus 24 \
  --max_memory 128.GB
```

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1215/

Name
..
apptainer_cache
assets
bin
conf
db
dockerfiles
docs
input
lib
matplotlib_cache
modules
output
results
sanibeloutput-20241030193223
subworkflows
work
work_prepare_db
workflows
bb_prepare_db.20303654.err
bb_prepare_db.20303654.out
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CODE_OF_CONDUCT.md
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main.nf
modules.json
nextflow.config
nextflow_schema.json
prepare_bigbacter_db.sh
pyproject.toml
README.md
run_bigbacter.sh
samplesheet.csv
tower.yml

sbatch
prepare_bigbacter_db.sh

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Application Cont.

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1215/

Name

- ..
- apptainer_cache
- assets
- bin
- conf
- db
- dockerfiles
- docs
- input
- lib
- matplotlib_cache
- modules
- output
- results
- sanibeloutput-20241030193223
- subworkflows
- work
- work_prepare_db
- workflows
- bb_prepare_db.20303654.err
- bb_prepare_db.20303654.out
- bigbacter.20353854.err
- bigbacter.20353854.out
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- nextflow_schema.json
- prepare_bigbacter_db.sh
- pyproject.toml
- README.md
- run_bigbacter.sh
- samplesheet.csv
- tower.yml

/blue/bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1215/output/1764971800/

Name

- ..
- Escherichia_coli
- other
- 1764971800-db-info.csv
- 1764971800-summary.tsv

ID	STATUS	QUAL	RUN_ID	TAXA	CLUSTER	ISO_IN_CLUSTER	ISO_PASS_QC	MEAN_SNP_DIST_SNIPPY	MIN_SNP_DIST_SNIPPY	MAX_SNP_DIST_SNIPPY	STRONG_LINKAGE_SNIPPY	INTER_LINKAGE_SNIPPY	MEAN_SNP_DIST_GUBBINS	MIN_SNP_DIST_GUBBINS	MAX_SNP_DIST_GUBBINS	STRONG_LINKAGE_GUBBINS	INTER_LINKAGE_GUBBINS	LENGTH	ALIGNED	UNALIGNED	RECOMB	VARIANT	HET	MASKED	LOWCOV	PER_GENFRAC	PER_LOWCOV	PER_HET
J7_T1	NEW	PASS	1.765E+09	Escherichia_coli	2	2	2	3510	9	7011	Reference	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	2022	not performec	9	#####	0	2353	99.5116	0.0447891	0.40508
J8_T1	NEW	PASS	1.765E+09	Escherichia_coli	2	2	2	7132	7011	7252	none	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	324318	not performec	7020	#####	0	20399	92.9468	0.388292	0.49154
Reference	OLD	PASS	1.765E+09	Escherichia_coli	2	2	2	3630	9	7252	J7_T1	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	0	not performec	0	0	0	0	100	0	0
J4_T1	NEW	PASS	1.765E+09	Escherichia_coli	5	2	2	5782	4	11560	Reference	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	425	not performec	4	#####	0	763	99.6074	0.0146395	0.36977
J52_T1	NEW	PASS	1.765E+09	Escherichia_coli	5	2	2	11672	11560	11784	none	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	659114	not performec	11564	#####	0	22001	86.5754	0.422129	0.35618
Reference	OLD	PASS	1.765E+09	Escherichia_coli	5	2	2	5894	4	11784	J4_T1	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	0	not performec	0	0	0	0	100	0	0
J17_T1	NEW	PASS	1.765E+09	Escherichia_coli	6	1	1	46	46	46	none	Reference	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	3136	not performec	46	#####	0	3076	99.4795	0.0584237	0.40251
Reference	OLD	PASS	1.765E+09	Escherichia_coli	6	1	1	46	46	46	none	J17_T1	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	0	not performec	0	0	0	0	100	0	0
J100_T1	NEW	PASS	1.765E+09	Escherichia_coli	12	1	1	2	2	2	Reference	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	346	not performec	2	#####	0	695	99.622	0.0143087	0.35656
Reference	OLD	PASS	1.765E+09	Escherichia_coli	12	1	1	2	2	2	J100_T1	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	0	not performec	0	0	0	0	100	0	0
J9_T1	NEW	PASS	1.765E+09	Escherichia_coli	627	1	1	3	3	3	Reference	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	62	not performec	3	#####	0	440	99.6499	0.00942095	0.33939
Reference	OLD	PASS	1.765E+09	Escherichia_coli	627	1	1	3	3	3	J9_T1	none	not performed	not performed	not performed	not performed	not performed	5E+06	5E+06	0	not performec	0	0	0	0	100	0	0

```
sbatch run_bigbacter.sh
```

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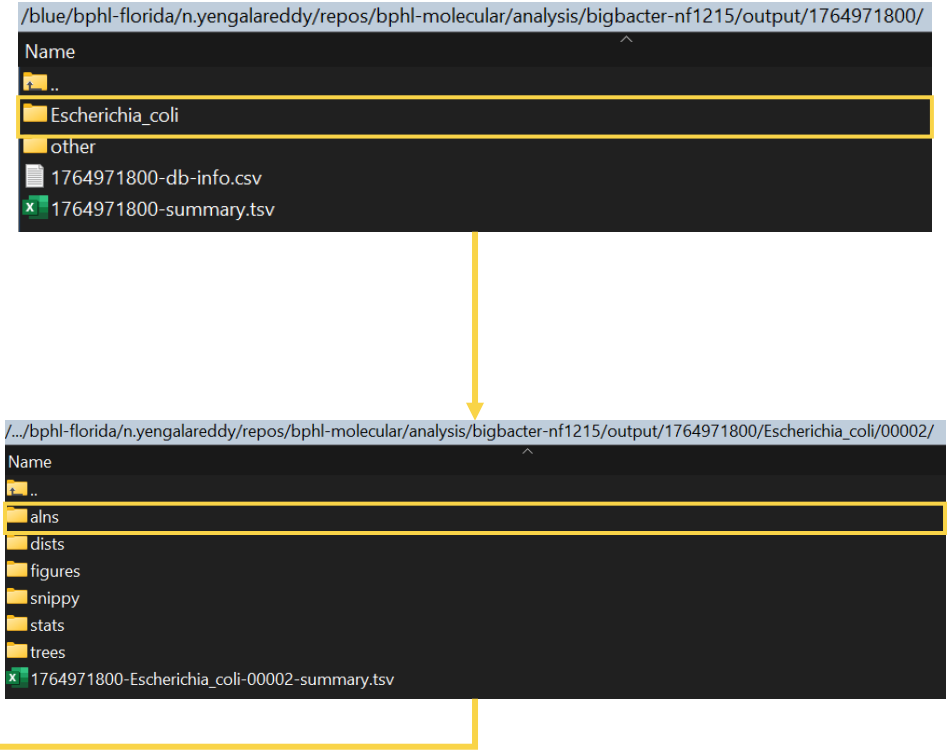
Application Cont.



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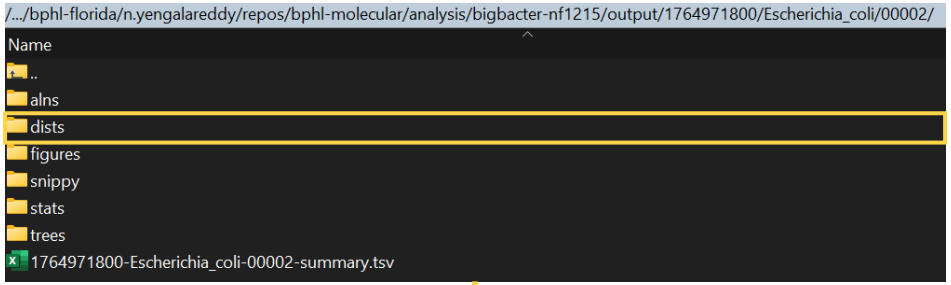
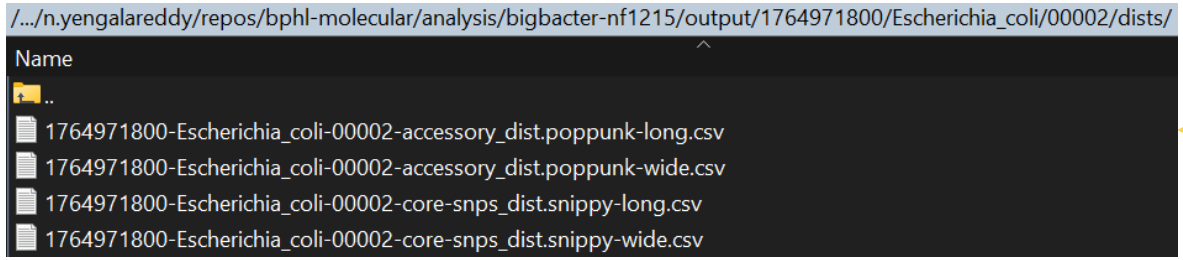
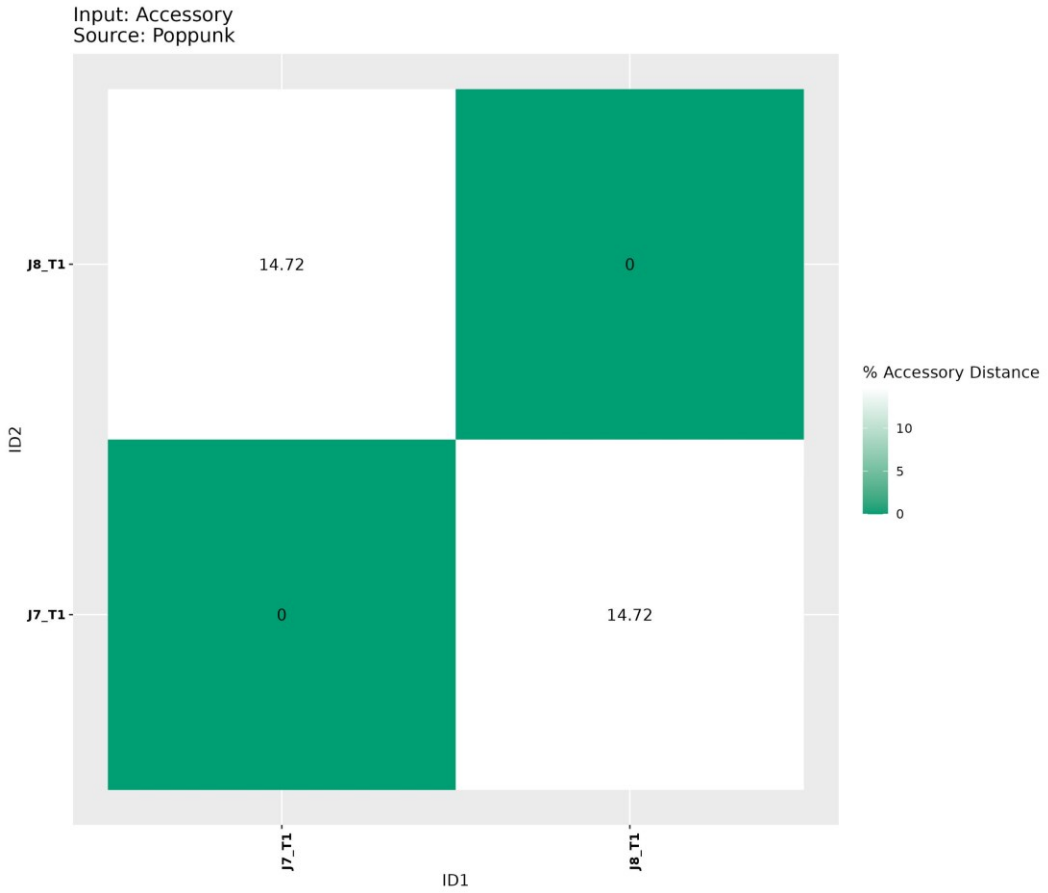
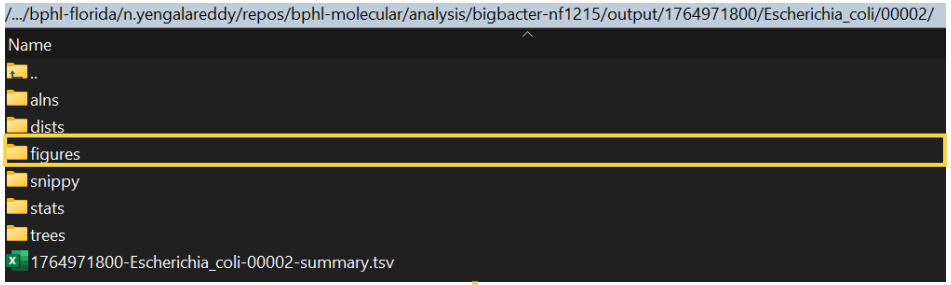
Application Cont.

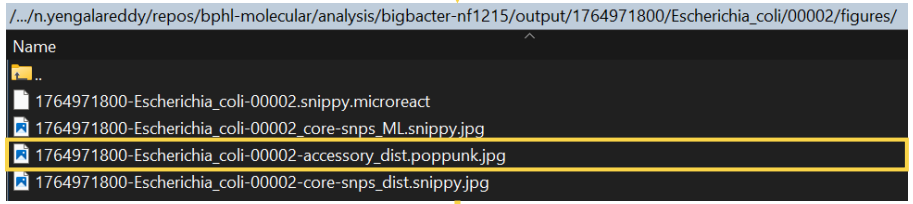




Application Cont.







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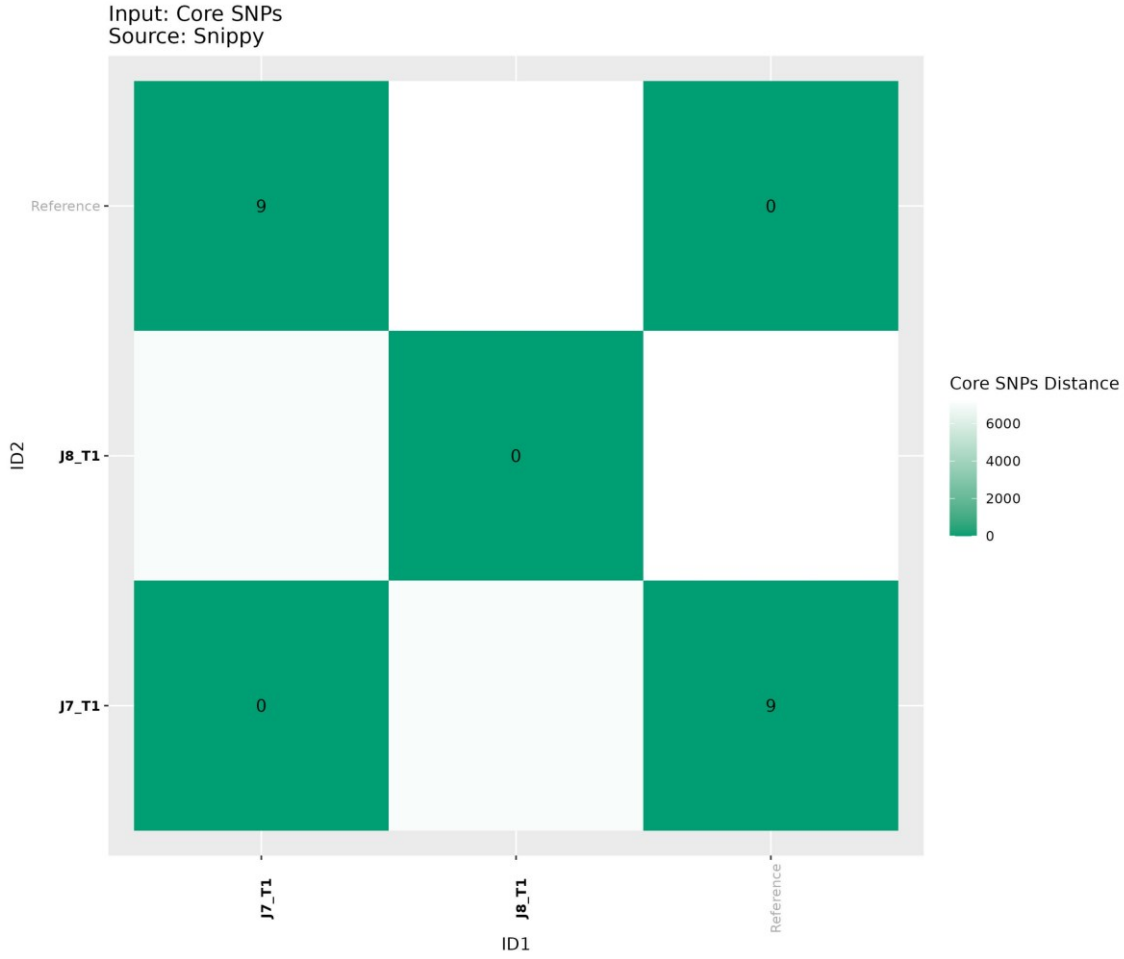
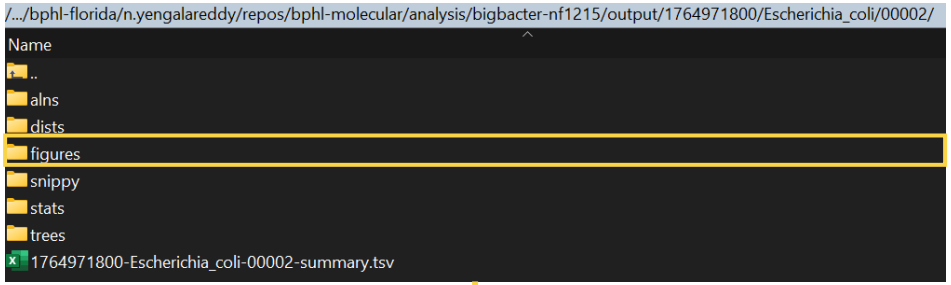
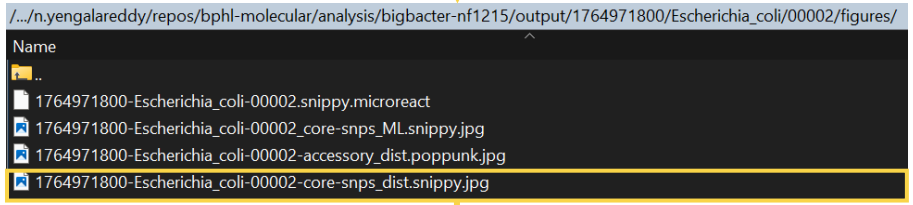
Application Cont.







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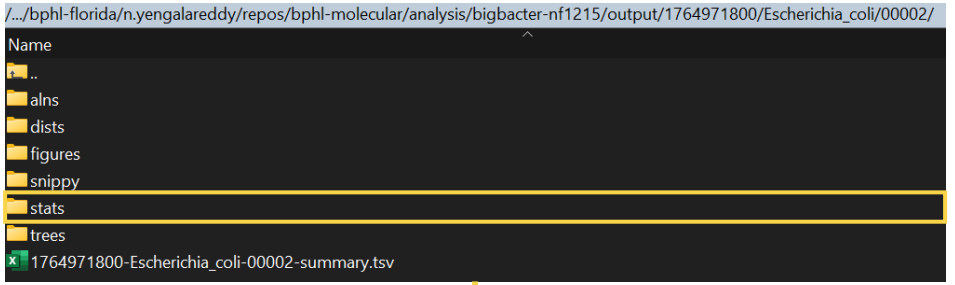
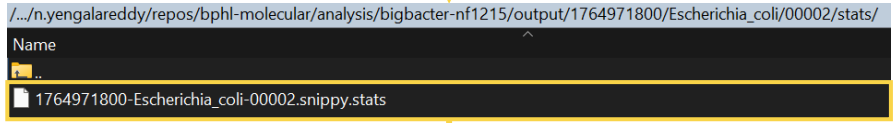
Application Cont.





ID	LENGTH	ALIGNED	UNALIGNED	VARIANT	HET	MASKED	LOWCOV	PER_GENFRAC	PER_LOWCOV	PER_HET	QUAL
J7_T1	5253514	5227858	2022	9	21281	0	2353	99.5116	0.0447891	0.405081	PASS
J8_T1	5253514	4882974	324318	7020	25823	0	20399	92.9468	0.388292	0.491538	PASS
Reference	5253514	5253514	0	0	0	0	0	100	0	0	PASS

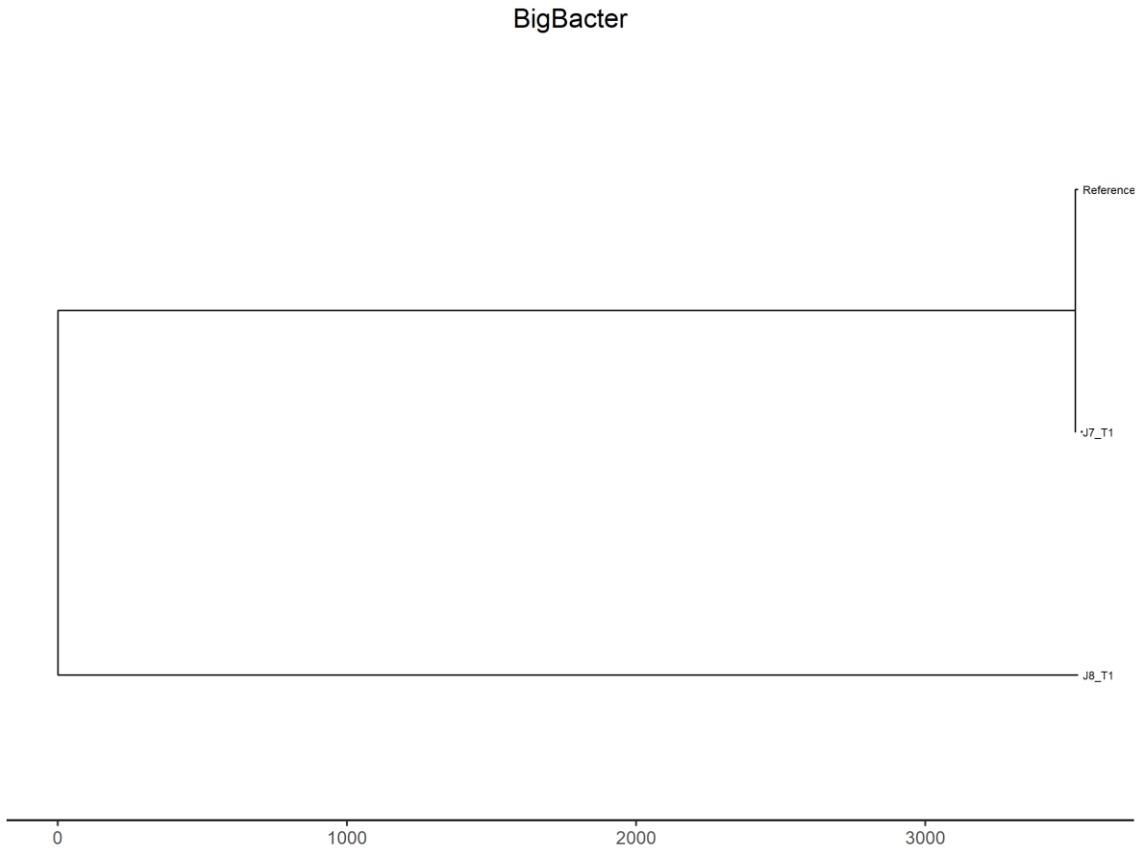
Application Cont.

/.../bphl-florida/n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1215/output/1764971800/Escherichia_coli/00002/

Name
..
alns
dists
figures
snippy
stats
trees
1764971800-Escherichia_coli-00002-summary.tsv

/.../n.yengalareddy/repos/bphl-molecular/analysis/bigbacter-nf1215/output/1764971800/Escherichia_coli/00002/trees/

Name
..
1764971800-Escherichia_coli-00002_core-snps_ML.snippy.final.nwk
1764971800-Escherichia_coli-00002_core-snps_ML.snippy.nwk



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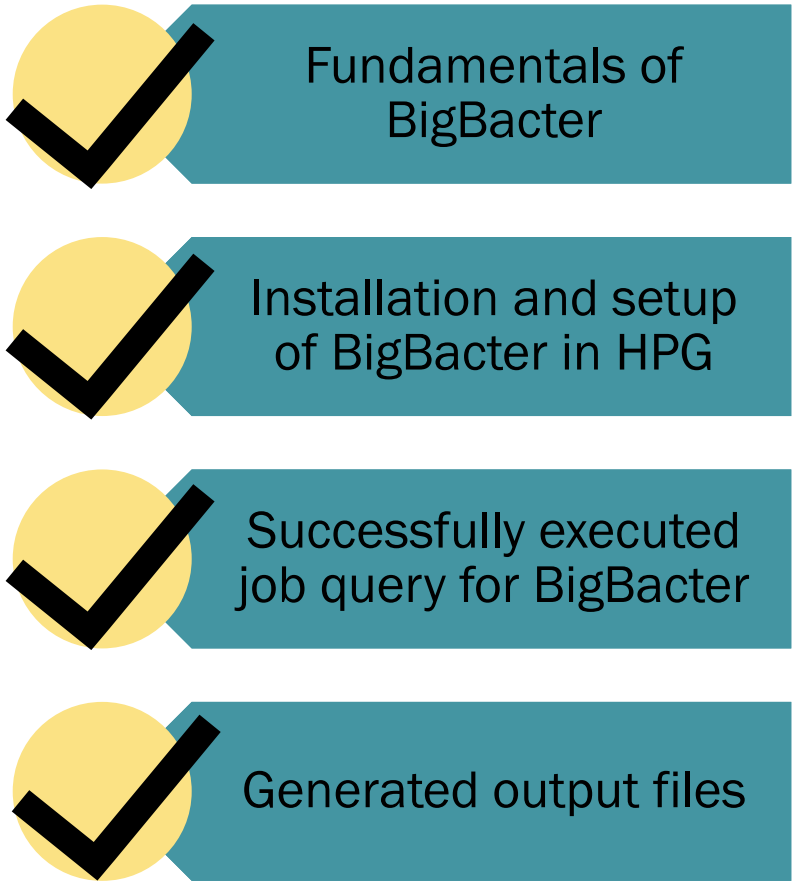
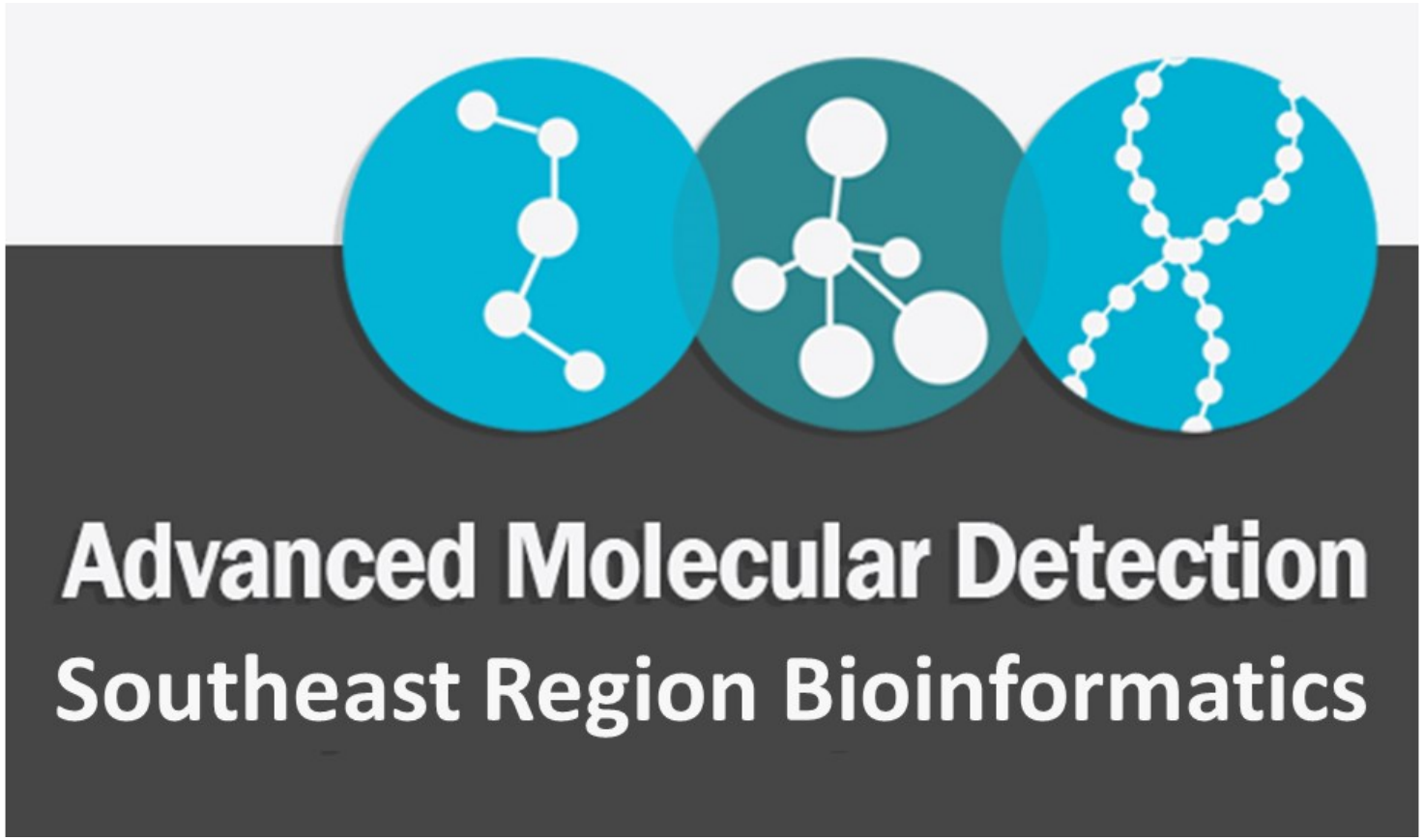
Conclusion


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



Questions?

bphl-sebioinformatics@flhealth.gov

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