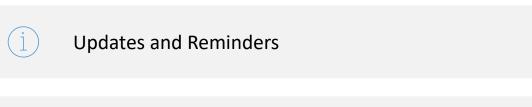
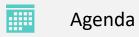
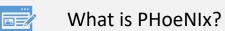


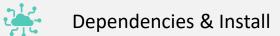
**Advanced Molecular Detection Southeast Region Bioinformatics** 

### Outline









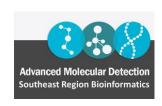
Testing Install & Running PHoeNIx

Output File Structure & Pipeline Summary

Questions

### Updates and Reminders

- Please email us on <u>bphl-sebioinformatics@flhealth.gov</u> for any queries or requests.
- One of the group members will respond to your query.



# Agenda

**April 24** – PHoeNIx #1 (Installation and Dependencies)

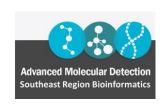
May 1 – PHoeNIx #2 (Demo & Troubleshooting)

May 15 – Open OnDemand

May 29 – AMRFinder+ and Pha4ge's hAMRonization pipeline

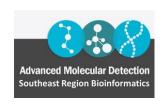
#### Future Trainings

- ONT & FL's Flisochar pipeline
- StaPH-B Toolkit Programs/Pipelines
- GISAID flagged SARS-CoV-2
- Git (git clone, etc.)
- Generating R figures
- Outbreak/cluster training
- SRA human scrubber tool
- ...and more



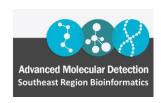
### What is PHoeNIx?

- PHoeNIx Portable Healthcare Nextgen Informatics pipeline
- Developed by the Division of Healthcare Quality Promotion (DHQP) at the CDC for pathogens commonly encountered in healthcare settings (including but not limited to e.g., Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus)
- Pipeline is built using Nextflow which runs tasks across multiple compute infrastructures in a portable manner
- Uses Docker/Singularity containers making installation trivial and highly reproducible results
- Pipeline provides a standardized approach for identifying and characterizing healthcareassociated bacterial pathogens, specifically for public health partners
- Nextflow DSL2 implementation of this pipeline uses one container per process which makes it easier to maintain and update software dependencies



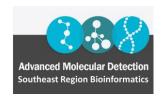
# PHoeNIx Pipeline Insights

- PHoeNIx uses Illumina paired-end reads and was designed for use with pathogens causing healthcareassociated bacterial infections. This comprehensive pipeline performs:
  - 1. Quality control
  - 2. Checks for contamination
  - 3. Confirms taxa ID
  - 4. Performs sequence typing
  - 5. Assembles reads into scaffolds
  - 6. Detects antibiotic resistance & hypervirulence genes
  - 7. Searches for plasmid markers
- PHoeNIx generates several files that are compatible with downstream analytic tools, such as those used for phylogenetic tree building
- This pipeline is available to run on Terra, Nextflow tower, CLI and is also incorporated into the StaPH-B toolkit



# Dependencies & Install

- Install Nextflow with conda-forge channel and a bioconda channel using mamba
- Configure Nextflow for command-line interface (CLI) users
- Install container software
- Transfer Kraken2 database files to a new directory using WinSCP
- Edit configuration files to run on HPG



### Install Nextflow

#### Install Nextflow environment using mamba:

1. Activate conda/mamba

\$ module load conda

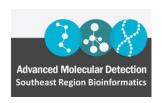
2. Install Nextflow

\$ mamba create -yp /blue/<bphl-state>/<user>/conda\_envs/nextflow -c conda-forge -c bioconda nextflow=21.10.6

3. Activate the mamba environment

\$ mamba activate /blue/<bphl-state>/<user>/conda\_envs/nextflow

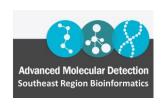
4. Then, run PHoeNIx from this environment



# Configuring Nextflow for CLI users

Configuration is done in the form of a config file & is needed so that Nextflow knows how to fetch the required software. Options include:

- Pipeline comes with config files called docker & singularity which instruct the pipeline to use the named tool for software management. For example, -profile test, singularity
- Check nf-core/configs to see if a custom config file already exists for your institute, so you can use -profile
   <institute> in your command which enables either docker or singularity to set the execution for your local compute environment
- Use --singularity\_pull\_docker\_container if you have issues downloading Singularity images. This will pull and convert the docker image instead.
- Alternatively, you can use **nf-core download** to download images first, before running the pipeline.
  - Set NXF\_SINGULARITY\_CACHEDIR or singularity.cachedir for Nextflow to store & reuse the images from a central location for future pipelines



# Configuration set up

To add NXF\_SINGULARITY\_CACHEDIR to your bash profile run the following:

Open your ~/.bash\_profile

\$ emacs ~/.bash\_profile

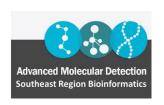
Add these lines within ~/.bash profile

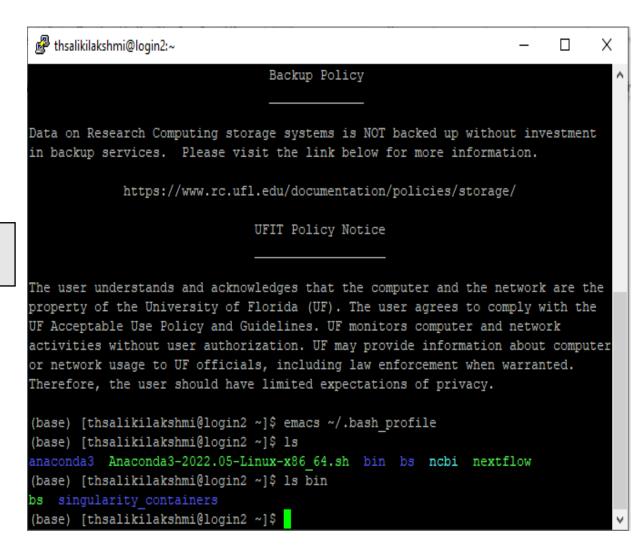
\$ export NXF\_SINGULARITY\_CACHEDIR=/\$PATH/Singularity\_containers export /path/to/singularity\_containers

 \$ /path/ is the full path of the folder where you want to store. (Singularity\_Containers can be named whatever you want)

\$ source ~/.bash\_profile

This command allows Nextflow to see the new path





# emacs ~/.bash\_profile

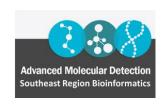
```
thsalikilakshmi@login2:~
                                                                                 Х
File Edit Options Buffers Tools Sh-Script Help
if [ -f ~/.bashrc ]; then
        . ~/.bashrc
fi
PATH=$PATH:$HOME/bin
export NXF SINGULARITY CACHEDIR=/$HOME/bin/singularity containers
export PATH
-UU-:---Fl .bash profile
                             All Ll
                                         (Shell-script[bash])
Indentation setup for shell type bash
```



### Download Database Files for Kraken2

- Email <u>HAISeq@cdc.gov</u>, with the subject line **"krakenDB invite request"** to request access to the sharefile link & provide the email address to send invite to
- Then, download the hash.k2d, opts.k2d, and taxo.k2d files needed for the Kraken2 subworkflow of PHoeNIx from the CDC sharefile link
- Other kraken2 databases cannot be used there is a specific **ktax\_map.k2** file needed for the pipeline
- Download via WinSCP
- Once downloaded, the folder containing these files should be passed to PHoeNIx via by
   --kraken2db

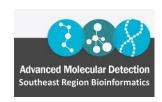
### We've shared the database files via HPG public-share



### Git Clone PHoeNIx

Install the latest version via cloning PHoeNIx GitHub repo into a folder of your choosing

```
$ cd $/path/to/local/phoenix/repo/
$ git clone https://github.com/CDCgov/phoenix
```



# Testing Install

 Test that the pipeline is installed and configured correctly:

```
$ nextflow run phoenix/main.nf –profile test,singularity --entry PHOENIX --kraken2db $PATH_TO_DB
```

- This command will run the pipeline on preloaded data
- If all goes well, the output will look like the screen on the right
- As seen from the image, pipeline takes ~21 mins
- Note: some steps aren't run in this pipeline (i.e., SPADES\_WF stats), this is normal.

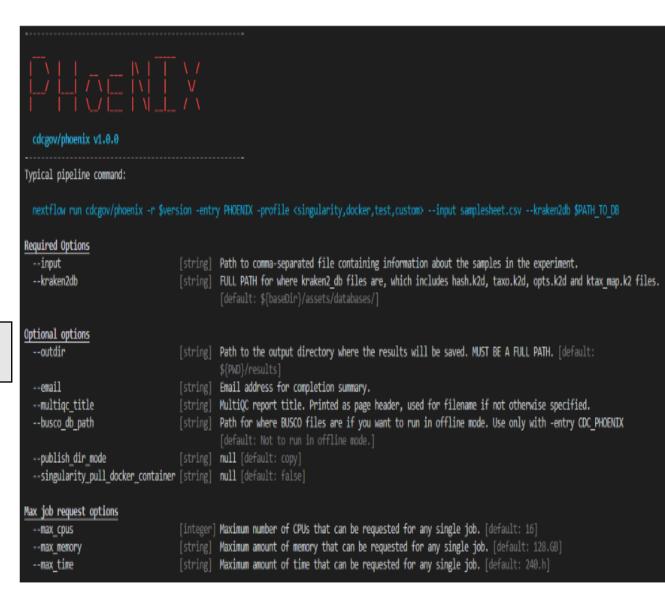
```
Advanced Molecular Detection
Southeast Region Bioinformatics
```

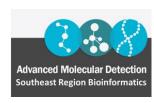
```
bb/5232b5] process > PHOENIX:PHOENIX EXTERNAL:BBMAP REFORMAT (Test Sample)
                                                                                                                [100%] l of l â
f/74bbc8] process > PHOENIX:PHOENIX EXTERNAL:MLST (Test Sample)
                                                                                                                [100%] 1 of 1 â
a3/a0512c1 process > PHOENIX:PHOENIX EXTERNAL:GAMMA HV (Test Sample)
98/2ad711] process > PHOENIX:PHOENIX EXTERNAL:GAMMA AR (Test Sample)
d9/c70240) process > PHOENIX:PHOENIX EXTERNAL:GAMMA PF (Test Sample)
75/4ed988] process > PHOENIX:PHOENIX EXTERNAL:QUAST (Test Sample)
e2/a6delb] process > PHOENIX:PHOENIX EXTERNAL:KRAKEN2 WTASMBLD:KRAKEN2 WTASMBLD (Test Sample)
0/999cfl] process > PHOENIX:PHOENIX EXTERNAL:KRAKEN2 WTASMBLD:KRAKENTOOLS MAKEKREPORT (Test Sample)
ld/55250a] process > PHOENIX:PHOENIX EXTERNAL:KRAKEN2 WTASMBLD:KREPORT2KRONA WTASMBLD (Test Sample)
                                                                                                                [100%] 1 of 1 â
fd/e217c8] process > PHOENIX:PHOENIX EXTERNAL:KRAKEN2 WTASMBLD:KRAKEN2 BH WTASMBLD (Test Sample)
                                                                                                                [100%] 1 of 1 â
d/ba5lcfl process > PHOENIX:PHOENIX EXTERNAL:KRAKEN2 WTASMBLD:KRONA KTIMPORTTEXT WTASMBLD (Test Sample)
                                                                                                                [100%] 1 of 1 â
df/9befae] process > PHOENIX:PHOENIX EXTERNAL:MASH DIST (Test Sample)
                                                                                                                [100%] 1 of 1 â
22/7f9290] process > PHOENIX:PHOENIX EXTERNAL:DETERMINE TOP TAXA (Test Sample)
                                                                                                                [100%] 1 of 1 â
Ba/c37688] process > PHOENIX:PHOENIX EXTERNAL:FASTANI (Test Sample)
d/llbld6| process > PHOENIX:PHOENIX EXTERNAL:FORMAT ANI (Test Sample)
2d/841f2a] process > PHOENIX:PHOENIX EXTERNAL:DETERMINE TAXA ID (Test Sample)
 7/da8448] process > PHOENIX:PHOENIX EXTERNAL:PROKKA (Test Sample)
[1/8bb531] process > PHOENIX:PHOENIX EXTERNAL:AMRFINDERPLUS UPDATE (update)
33/2777dl] process > PHOENIX:PHOENIX EXTERNAL:GET TAXA FOR AMRFINDER (Test Sample)
7b/f699al] process > PHOENIX:PHOENIX EXTERNAL:AMRFINDERPLUS RUN (Test Sample)
                                                                                                                [100%] 1 of 1 â
e/alc3f4] process > PHOENIX:PHOENIX EXTERNAL:CALCULATE ASSEMBLY RATIO (Test Sample)
f3/5854fa] process > PHOENIX: PHOENIX EXTERNAL: GENERATE PIPELINE STATS WF: GENERATE PIPELINE STATS (Test Sample) [100%] 1 of 1 â
13/dd23cc] process > PHOENIX:PHOENIX EXTERNAL:CREATE SUMMARY LINE (Test Sample)
e4/42dd47] process > PHOENIX:PHOENIX EXTERNAL:FETCH FAILED SUMMARIES
c9/011ce8] process > PHOENIX:PHOENIX EXTERNAL:GATHER SUMMARY LINES (1)
                                                                                                                [100%] 1 of 1 â
f5/b965e6] process > PHOENIX:PHOENIX EXTERNAL:CUSTOM DUMPSOFTWAREVERSIONS (1)
                                                                                                                [100%] 1 of 1 â
[0/3f52ae] process > PHOENIX:PHOENIX EXTERNAL:MULTIQU
                                                                                                                [100%] 1 of 1 â
        phoenix| Pipeline completed successfully-
**Reminder: If your lab has received funds (ELC, SHARP, etc.) to sequence isolates under the HAI/AR component of the AR Lab Network, please upload rele
nt sequence files to CDC's NCBI HAI-Seq Umbrella Project (ID 531911) and update any relevant alerts records with the HAI WGS ID and SRR ID, within 7-10
 siness days from sequencing completion.***
 N: To render the execution DAG in the required format it is required to install Graphviz -- See http://www.graphviz.org for more info.
   leted at: 31-Jan-2023 08:24:08
          : 27m 48s
/blue/bphl-florida/thsalikilakshmi/training/conda envs/nextflow) [thsalikilakshmi@c0704a-s2 training]$ nextflow run cdcgov/phoenix -r vl.0.0 -profile
 t, singularity -entry PHOENIX --kraken2db /blue/bphl-florida/thsalikilakshmi/training/pheonix testing/
```

### Running PHoeNIx

The help command will display required and optional options

nextflow run /blue/<bphl-state>/<user>/phoenix/phoenix help





### Inputs

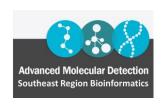
- PHoeNIx runs only on Illumina paired-end reads
- Multiple samples can be analyzed using a samplesheet.csv file

nextflow run cdcgov/phoenix -profile singularity -entry PHOENIX --input samplesheet.csv --kraken2db \$PATH\_TO\_DB

#### **Sample Sheet Input:**

--input '[path to sample sheet file]'

- Sample sheet to be created with the information about the samples to be analyzed before running the pipeline
- Use --input parameter to specify the location



# Sample Sheet Input

- Sample sheet must be a comma-separated file (.csv) with only 1 column and a header row
- DO NOT HAVE ANY SPACES IN THIS FILE
- Make sure the paths are full paths and not relative
- Automated sample sheet also can be created
  - This will be shown in a few slides
- Final sample sheet file consisting of pairedend data looks something like the image on the right

#### sample,fastq\_1,fastq\_2

JBI22000743,/blue/bphl-

florida/thsalikilakshmi/data/HAI/20220829\_jax\_220823\_PLN\_WLK\_MS/fastqs/JBI22000743\_1.fastq.gz,/blue/bphl-florida/thsalikilakshmi/data/HAI/20220829\_jax\_220823\_PLN\_WLK\_MS/fastqs/JBI22000743\_2.fastq.gz

JBI22000770,/blue/bphl-

florida/thsalikilakshmi/data/HAI/20220829\_jax\_220823\_PLN\_WLK\_MS/fastqs/JBI22000770\_1.fastq.gz,/blue/bphl-florida/thsalikilakshmi/data/HAI/20220829\_jax\_220823\_PLN\_WLK\_MS/fastqs/JBI22000770\_2.fastq.gz

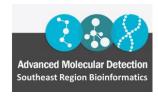
JBI22000771,/blue/bphl-

florida/thsalikilakshmi/data/HAI/20220829\_jax\_220823\_PLN\_WLK\_MS/fastqs/JBI22000771\_1.fastq.gz,/blue/bphl-florida/thsalikilakshmi/data/HAI/20220829\_jax\_220823\_PLN\_WLK\_MS/fastqs/JBI22000771\_2.fastq.gz



# Sample Sheet Description

| Column  | Description   |
|---------|---|
| sample  | Custom sample name. This entry will be identical for multiple sequencing libraries/runs from the same sample. Spaces in sample names are automatically converted to underscores (_) |
| fastq_1 | Full path to <i>fastq</i> file for Illumina short reads 1. File has to be gzipped and have the extension ".fastq.gz" or ".fq.gz"  |
| fastq_2 | Full path to <i>fastq</i> file for Illumina short reads 2. File has to be gzipped and have the extension ".fastq.gz" or ".fq.gz"  |

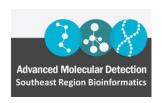


### Automated Sample Sheet Creation

 The sample sheet can be created automatically using the below command from a directory of fastq files

phoenix/bin/create\_samplesheet.sh <directory of fastq files> > <output\_directory>/samplesheet.csv

- The script will search 1 directory deep & attempt to determine sample id names and pairing/multilane information to create a sample sheet automatically
- Please review the sample sheet for accuracy before using it in the pipeline
- samplesheet.csv can be changed to your preferred name



### Outputs

#### ANI

- Developed for fast alignment-free computation of whole-genome Average Nucleotide Identity (ANI)
- Avoids expensive sequence alignments and uses Mashmap as its MinHash based sequence mapping engine to compute the orthologous mappings and alignment identity estimates

#### **AMRFinder**

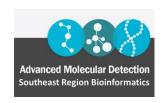
• AMRFinder and the accompanying database identify acquired antimicrobial resistance genes in bacterial protein and/or assembled nucleotide sequences as well as known resistance-associated point mutations for several taxa

#### **Assembly**

- SPAdes St. Petersburg genome assembler is an assembly toolkit containing various assembly pipelines
- SPAdes scaffold files are used for downstream analysis.

#### **BUSCO** (only run with --entry CDC\_PHOENIX)

 BUSCO output is based on evolutionarily-informed expectations of gene content of near-universal single-copy orthologs, thus the BUSCO metric is complementary to technical metrics like N50



### Outputs

#### fastp

• A tool designed to provide fast all-in-one preprocessing (trimming) of *fastq* files. This tool was developed in C++ with multithreading supported to afford high performance

#### **FastQC**

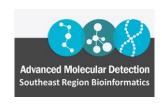
- Provides general quality metrics about your sequenced reads
- Provides information about the quality score distribution across your reads, per base sequence content (%A/T/G/C), adapter contamination, and overrepresented sequences

#### **GAMMA**

• GAMMA (Gene Allele Mutation Microbial Assessment) is a command line tool that finds gene matches in microbial genomic data using protein coding (rather than nucleotide) identity then translates and annotates the match by providing the type (i.e., mutant, truncation, etc.) and a translated description (i.e., Y190S mutant, truncation at residue 110, etc.)

#### Kraken2

- A taxonomic classifier using exact k-mer matches to achieve high accuracy and fast classification speeds
- This classifier matches each k-mer within a query sequence to the lowest common ancestor (LCA) of all genomes containing the given k-mer



### Outputs

#### **MLST**

Scans assembly files against traditional PubMLST typing schemes

#### **QUAST**

Evaluates the quality of genome assemblies

#### removedAdapters

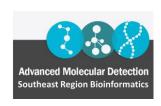
• BBDUK was developed to combine most common data-quality-related trimming, filtering, and masking operations into a single high-performance tool

#### SRST2 (only run with --entry CDC\_PHOENIX)

Short Read Sequence Typing for Bacterial Pathogens

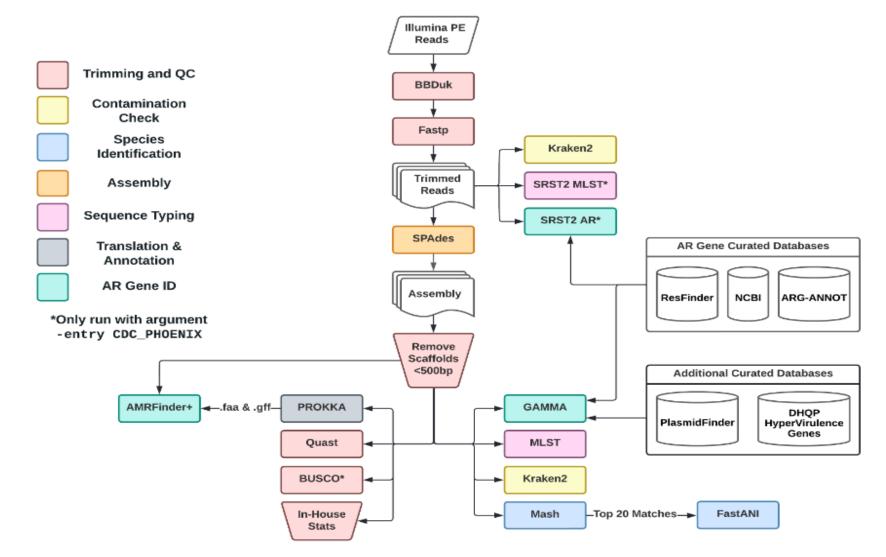
#### **MultiQC**

- A visualization tool that generates a single HTML report summarizing all samples in your project
- Results generated by MultiQC collate pipeline QC from supported tools e.g., FastQC



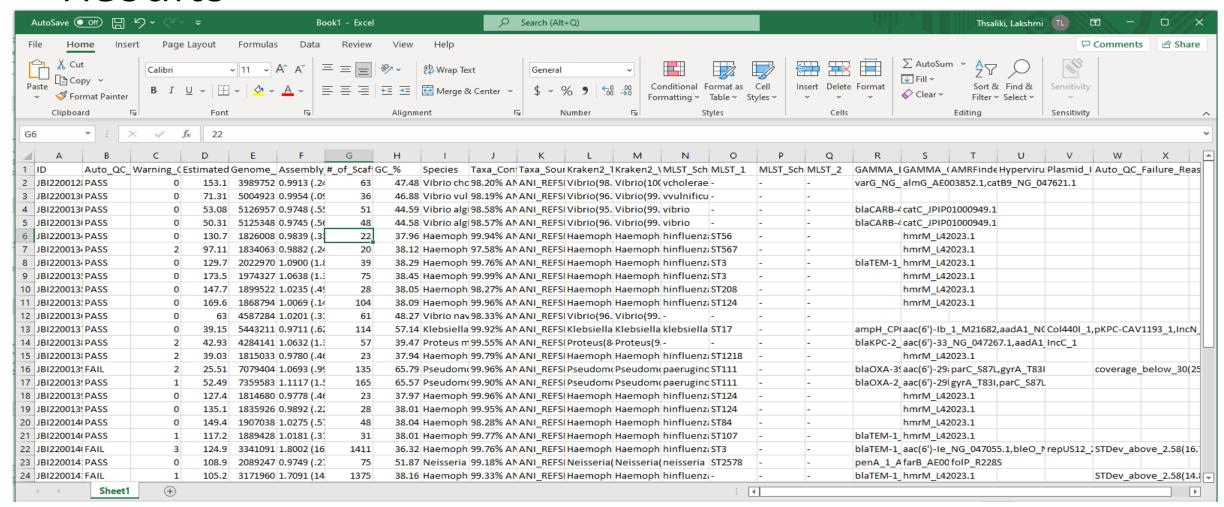
# Pipeline Summary

#### PHoeNIx v1.0.0 Workflow





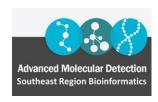
### Results





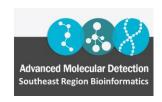
### Link to PHoeNIx

GitHub link for CDC PHoeNIx pipeline: <a href="https://example.com/bhoenix-wiki-GitHub">Home · CDCgov/phoenix Wiki · GitHub</a>



### Next Week

- We'll go over editing the HPC config file for running on HPG
- We'll have a quick demo and time for questions and troubleshooting



### Time for Questions & Feedback

- Questions?
  - Do you need help with anything?
  - Requests for separate trainings?
- Feedback
  - What would you like to see?





# **Advanced Molecular Detection Southeast Region Bioinformatics**

**Questions?** 

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