



Advanced Molecular Detection

Southeast Region Bioinformatics

Outline



Updates



Agenda



Kleborate



Legsta



Questions

Updates – ABiL Trainings

ABiL in-person courses

- The ABiL in-person (advanced!) bioinformatics course will be held **Tuesday Oct. 24th - Friday Oct. 27th** at the Georgia Institute of Technology (GT)
- About 2 participants may attend from each state or jurisdiction
- Tanya has sent an email about the courses
- Email us ASAP if you're interested to guarantee a spot!

ABiL online courses

- If additional attendees decide to sign up later, that is not a problem, as the courses will still be available, and they can attend on a rolling basis under the contract
- If you did not receive the invitation for joining the courses, please let us know, we can help to get you enrolled

Agenda

October 2 – Bactopia Tools: LisSero and Meningotype

October 16 – Bactopia Tools: Mcroni and MOB_suite

Future Trainings

- ONT & FL's Flisochar pipeline
- StaPH-B Toolkit Programs/Pipelines
- GISAID flagged SARS-CoV-2
- R Training Series
- Dryad pipeline
- ...and more

Kleborate

Kleborate is a tool to screen genome assemblies of *Klebsiella pneumoniae* and the *Klebsiella pneumoniae* species complex (KpSC) for:

- MLST
- Species (e.g. *K. pneumoniae*, *K. quasipneumoniae*, *K. variicola*, etc.)
- ICE*Kp* associated virulence loci: yersiniabactin (*ybt*), colibactin (*clb*), salmochelin (*iro*), hypermucoidy (*rmpA*)
- Virulence plasmid associated loci: salmochelin (*iro*), aerobactin (*iuc*), hypermucoidy (*rmpA*, *rmpA2*)
- Antimicrobial resistance determinants: acquired genes, SNPs, gene truncations and intrinsic β -lactamases
- K (capsule) and O antigen (LPS) serotype prediction, via *wzi* alleles and Kaptive

[klebgonomics/Kleborate \(github.com\)](https://github.com/klebgonomics/Kleborate)

Overview

- *Klebsiella pneumoniae* (Kp) is a commensal bacterium that causes opportunistic infections in hospitals. It has six close relatives (species and subspecies), and together these are known as the *K. pneumoniae* species complex (KpSC). These related species are often difficult to distinguish from one another in clinical labs using biotyping or MALDI-TOF and consequently can be confused for *K. pneumoniae* sensu stricto.
- Extracts clinically relevant genotyping information on *K. pneumoniae* and the KpSC using genome data
- Kleborate - a genomic surveillance tool is designed to:
 - Accurately identify species and sequence types
 - Identify the key acquired genetic features for which there is strong evidence of association with either antibiotic resistance or hypervirulence in *K. pneumoniae* sensu stricto
 - Predict K and O types

Installation

Can be installed through conda

```
conda create -yp /blue/bphl-<state>/<user>/conda_envs/kleborate/  
conda activate /blue/bphl-<state>/<user>/conda_envs/kleborate/  
conda install -c conda-forge -c bioconda kleborate
```

Usage

```
thsalikilakshmi@login2:/blue/bphl-florida/thsalikilakshmi/data/jbi/20220825_jax_220629_PLN_WLK_MS/fastqs
[thsalikilakshmi@login2 fastqs]$ conda activate /blue/bphl-florida/thsalikilakshmi/training/conda_envs/kleborate/
(/blue/bphl-florida/thsalikilakshmi/training/conda_envs/kleborate) [thsalikilakshmi@login2 fastqs]$ kleborate --help
usage: kleborate -a ASSEMBLIES [ASSEMBLIES ...] [-r] [--kaptive_k] [--kaptive_o] [-k] [--all] [-o OUTFILE]
               [--kaptive_k_outfile KAPTIVE_K_OUTFILE] [--kaptive_o_outfile KAPTIVE_O_OUTFILE]
               [--min_identity MIN_IDENTITY] [--min_coverage MIN_COVERAGE]
               [--min_spurious_identity MIN_SPURIOUS_IDENTITY] [--min_spurious_coverage MIN_SPURIOUS_COVERAGE]
               [--min_kaptive_confidence {None,Low,Good,High,Very_high,Perfect}] [--force_index] [-h]
               [--version]

Kleborate: a tool for characterising virulence and resistance in Klebsiella

Required arguments:
  -a ASSEMBLIES [ASSEMBLIES ...], --assemblies ASSEMBLIES [ASSEMBLIES ...]
                                     FASTA file(s) for assemblies

Screening options:
  -r, --resistance                  Turn on resistance genes screening (default: no resistance gene screening)
  --kaptive_k                       Turn on Kaptive screening of K loci (default: do not run Kaptive for K
                                     loci)
  --kaptive_o                       Turn on Kaptive screening of O loci (default: do not run Kaptive for O
                                     loci)
  -k, --kaptive                     Equivalent to --kaptive_k --kaptive_o
  --all                             Equivalent to --resistance --kaptive

Output options:
  -o OUTFILE, --outfile OUTFILE     File for detailed output (default: Kleborate_results.txt)
  --kaptive_k_outfile KAPTIVE_K_OUTFILE
                                     File for full Kaptive K locus output (default: do not save Kaptive K locus
                                     results to separate file)
  --kaptive_o_outfile KAPTIVE_O_OUTFILE
                                     File for full Kaptive O locus output (default: do not save Kaptive O locus
                                     results to separate file)
```



Input

- Kleborate takes *Klebsiella* genome assemblies in FASTA format (can be gzipped). It will work on either draft or completed assemblies, though completed is better because it reduces the risk of fragmented genes/loci

```
(/blue/bphl-florida/thsalikilakshmi/training/conda_envs/kleborate) [thsalikilakshmi@login2 fastqs]$ kleborate -o  
results_res_kaptive.txt --all -a /blue/bphl-  
florida/thsalikilakshmi/data/jbi/20220825_jax_220629_PLN_WLK_MS/assemblies/JBI22000619.fasta /blue/bphl-  
florida/thsalikilakshmi/data/jbi/20220825_jax_220629_PLN_WLK_MS/assemblies/JBI22000680.fasta
```

Results

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	strain	species	ST	virulence	resistance	Yersiniab	YbST	Colibactin	CbST	Aerobacti	AbST	Salmoche	SmST	RmpADC	RmST	rmpA2	wzi	K_locus	K_locus	O_locus	O_locus_c	AGly_a
2	JB122000619	Klebsiella pneumoniae	ST34	0	2 -		0 -		0 -		0 -		0 -		0 -		wzi162	unknown	None	O1/O2v2	Very high	aac(3)-I
3	JB122000680	Klebsiella pneumoniae	ST258	0	2 -		0 -		0 -		0 -		0 -		0 -		wzi154	unknown	None	O1/O2v2	Very high	aadA*

Legsta

- In silico *Legionella pneumophila* Sequence Based Typing (SBT)
- SBT stands for sequence-based typing. The purpose of the *Legionella pneumophila* SBT scheme is to provide a rapid and easily comparable method for the epidemiological typing of clinical and environmental isolates of *Legionella pneumophila* in outbreak investigations

tseemann/legsta:   In silico *Legionella pneumophila* Sequence Based Typing ([github.com](https://github.com/tseemann/legsta))

Installation

Can be installed through conda

```
conda create -yp /blue/bphl-<state>/<user>/conda_envs/legsta/  
conda activate /blue/bphl-<state>/<user>/conda_envs/legsta/  
conda install -c conda-forge -c bioconda legsta
```

Usage

```
(/blue/bph1-florida/thsalikilakshmi/training/conda_envs/legsta) [thsalikilakshmi@login5 conda_envs]$ legsta --help
NAME
  legsta 0.5.1
SYNOPSIS
  Legionella in silico SBT typing of contig sequences
USAGE
  legsta [options] <contigs.fa> ...
OPTIONS
  --help          This help.
  --version       Print version and exit.
  --quiet         Don't print anything to stderr.
  --debug+        Verbose debug output to stderr (default '0').
  --dbdir=s       SBT database folder (default '/blue/bph1-florida/thsalikilakshmi/training/conda_envs/legsta/db').
  --csv           Output CSV instead of TSV (default '0').
  --noheader      Don't print header row (default '0').
HOMEPAGE
  https://github.com/tseemann/legsta
```



Input

Accepts FASTA, Genbank, EMBL, GFF, and many other formats

```
legsta /blue/bphl-  
florida/thsalikilakshmi/data/legionella/20230719_jax_230531_PLN_WAT_JD/assemblies/JBI23001169.fasta > legsta_out
```

Results

- aFILE – name of the file
- SBT – sequence based typing
- flaA – flagellin gene
- pile – pilin gene
- asd – Aspartate-Beta- Semialdehyde Dehydrogenase gene
- mip – macrophage infection enhancer protein
- mompS – major outer membrane proteins
- proA – major secretory protein, mediates virulence regulation, host tissue degradation and immune evasion
- neuA – N-Acylneuraminate Cytidyltransferase Gene

	A	B	C	D	E	F	G	H	I
1	aFILE	SBT	flaA	pile	asd	mip	mompS	proA	neuA
2	/blue/bph	1	1	4	3	1	1	1	1



Advanced Molecular Detection Southeast Region Bioinformatics

Questions?

bphl-sebioinformatics@flhealth.gov

Lakshmi Thsaliki, MS

Bioinformatician

Lakshmi.Thsaliki@flhealth.gov

Molly Mitchell, PhD

Bioinformatician

Molly.Mitchell@flhealth.gov