BACTOPIA

COMPLETE ANALYSIS OF BACTERIAL GENOMES

Workshop for using Bactopia

CDC Enteric Diseases Laboratory Branch

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Wyoming Public Health LABORATORY

Disclaimer

The views and opinions expressed in today's workshop are mine and do not necessarily reflect the views or positions of the Wyoming Public Health Laboratory

Today’s Workshop Outline

Background

First, we’ll learn about Bactopia as a pipeline. We’ll investigate the many moving parts of Bactopia.

Bactopia

We’ll install Bactopia and process a few genomes through the main pipeline. During this time we will take a deeper look into a few steps.

Bactopia Tools

We’ll run Bactopia Tools to learn how these independent workflows can boost your analyses.

Outline for Bactopia Introductions

People

Meet the people behind Bactopia and how they are helping to improve it.

Bactopia

An introduction into Bactopia and how Bactopia Tools help streamline complex analyses.

Design Decisions

A quick glimpse into some decisions that were made ease on-going development.

Ehancements to OSS

Learn how Bactopia is helping to further enhance open-source science.

Future Directions

A look into what is on the horizon for Bactopia. Many new changes coming soon.

Wrap Up

Not much to say here, we'll close the first part of this session.

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People behind Bactopia

Let’s put some faces to Bactopia

Yo! 👋

I AM Robert

The developer and maintainer of Bactopia

Supporting Roles

Tim Read, PhD
Emory University
Professor

Tim has played a role in Bactopia since its inception. Through the years Tim has provided feedback and ideas to help shape Bactopia.

Joseph Reed, PhD
WPHL
Laboratory Administrator

As the WPHL lab administrator, Joe encourages the lab to pursue the development of skills and tools like Bactopia to strengthen WPHL.

Jim Mildenberger
WPHL
Molecular Lab Supervisor

Jim keeps the molecular lab running. Like Joe, Jim's support has helped introduce many new features (e.g., ONT support) into Bactopia.

Taylor Fearing
WPHL
EID & NGS Supervisor

Taylor oversees the sequencing lab at WPHL. She has played a tremendous and critical role in helping to expand Bactopia into public health.

Many scientists around the world that provide feedback

Wyoming Public Health Lab

15-20 people at WPHL

Led nation in % SC2+ cases sequenced

Domestic and International trainings

Strong relationship with Vet Lab at Ag Lab

> *My colleagues at the Wyoming Public Health Laboratory and Emory University have played an incredibly supportive role in the advancement of Bactopia.*

EMORY UNIVERSITY SCHOOL OF MEDICINE

Wyoming Public Health LABORATORY

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BACTOPIA

COMPLETE ANALYSIS OF BACTERIAL GENOMES

Let’s learn about the Bactopia and Bactopia Tools

> *In a few steps, Bactopia allows you to go from raw data to investigating your results*

What is Bactopia?

Bactopia is an extensive Nextflow pipeline for the complete analysis of bacterial genomes. To learn more, follow the step-by-step guide below.

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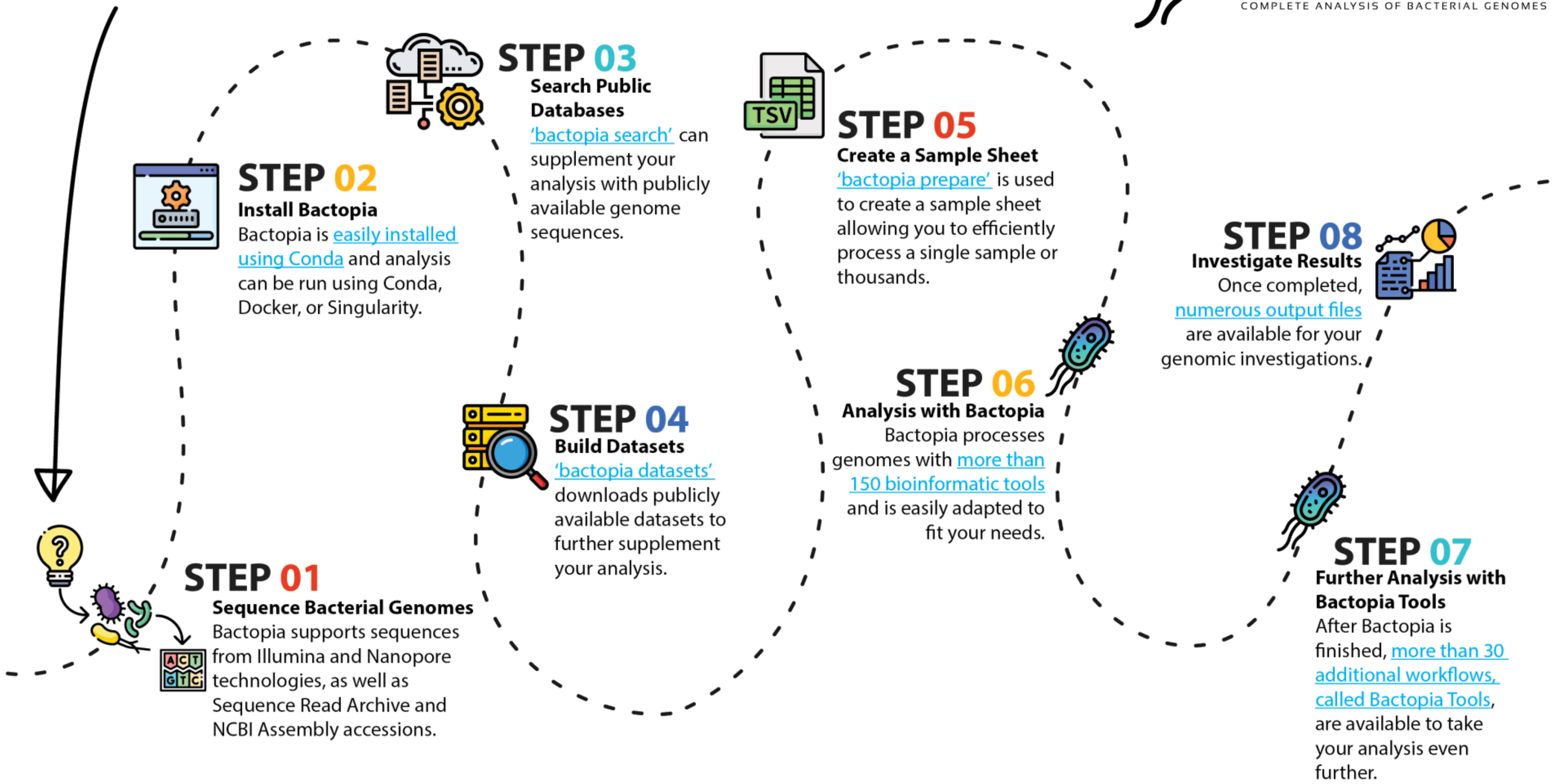
COMPLETE ANALYSIS OF BACTERIAL GENOMES



STEP 01
Sequence Bacterial Genomes
Bactopia supports sequences from Illumina and Nanopore technologies, as well as Sequence Read Archive and NCBI Assembly accessions.

STEP 02
Install Bactopia
Bactopia is easily installed using Conda and analysis can be run using Conda, Docker, or Singularity.

STEP 03
Search Public Databases
'bactopia search' can supplement your analysis with publicly available genome sequences.

STEP 04
Build Datasets
'bactopia datasets' downloads publicly available datasets to further supplement your analysis.

STEP 05
Create a Sample Sheet
'bactopia prepare' is used to create a sample sheet allowing you to efficiently process a single sample or thousands.

STEP 06
Analysis with Bactopia
Bactopia processes genomes with more than 150 bioinformatic tools and is easily adapted to fit your needs.

STEP 07
Further Analysis with Bactopia Tools
After Bactopia is finished, more than 30 additional workflows, called Bactopia Tools, are available to take your analysis even further.

STEP 08
Investigate Results
Once completed, numerous output files are available for your genomic investigations.

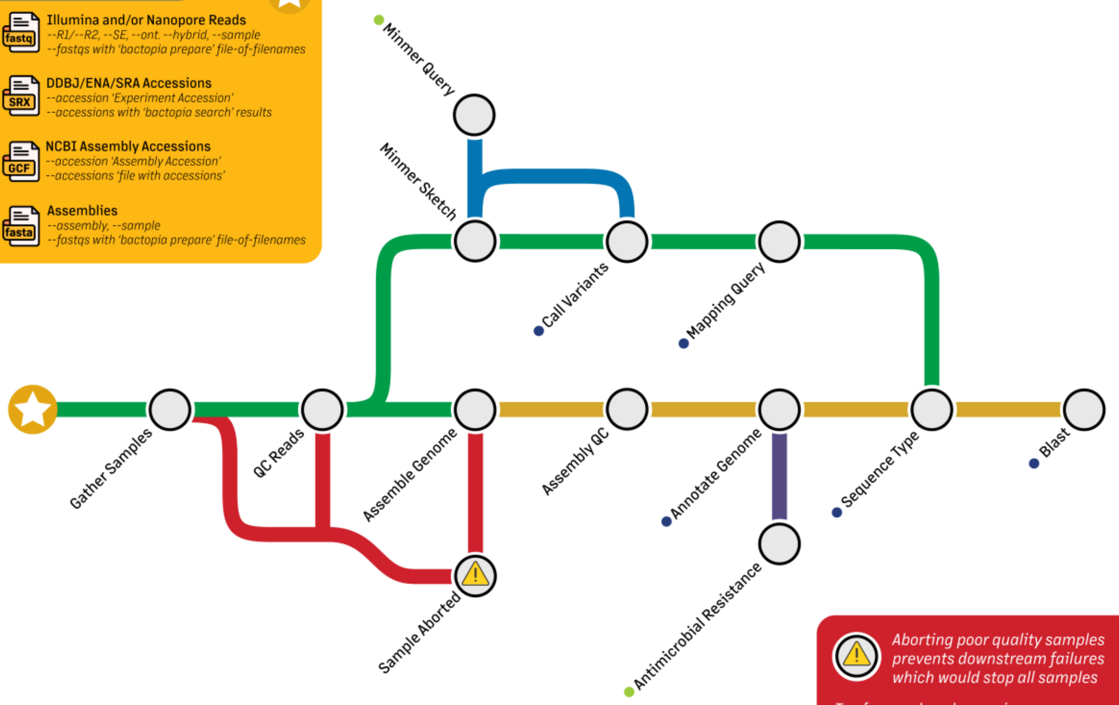
Accepted Inputs

Illumina and/or Nanopore Reads
--R1/--R2, --SE, --ont, --hybrid, --sample
--fastqs with 'bactopia prepare' file-of-filenames

DDBJ/ENA/SRA Accessions
--accession 'Experiment Accession'
--accessions with 'bactopia search' results

NCBI Assembly Accessions
--accession 'Assembly Accession'
--accessions 'file with accessions'

Assemblies
--assembly, --sample
--fastqs with 'bactopia prepare' file-of-filenames



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COMPLETE ANALYSIS OF BACTERIAL GENOMES

Aborting poor quality samples prevents downstream failures which would stop all samples

- Too few reads or basepairs
- Coverage below minimum
- Paired-end with mismatching IDs
- Paired-end with different read counts
- Paired-end with skewed proportions
- Genome size below minimum
- Genome size exceeds maximum
- 0 assembled contigs
- Assembled size below minimum

Abort Reasons

Legend

🟩	Process uses FASTQs
🟨	Process uses Contigs
🟦	Process uses Minmer Sketches
🟪	Process uses Contigs and Proteins
🟥	Minimum QC not met, sample aborted

Supplemented By Bactopia Datasets

●	Generic datasets (--datasets)
●	Species-specific datasets (--species)

Bactopia Processes

Gather Samples
Collect local files and/or download SRA or NCBI Assembly

QC Reads
Trim and filter low quality reads, subsample to specified coverage, and generate quality summary metrics

Minmer Sketch and Minmer Query
Create minmer sketches and query them against RefSeq and GenBank

Call Variants
Determine SNPs and InDels against a reference genome

Mapping Query
Align to a reference and determine per-base coverage

Assemble Genome & Assembly QC
Create a de novo assembly and summary metrics, then assess the quality of the assembly

Annotate Genome
Predict genes and proteins from the assembled contigs

Antimicrobial Resistance
Identify presence of AMR and/or virulence genes

Blast
Align genes, proteins, or primers to assembled contigs

Sequence Type
Determine sequence type base on PubMLST profiles

Bactopia Tools

More workflows for more science

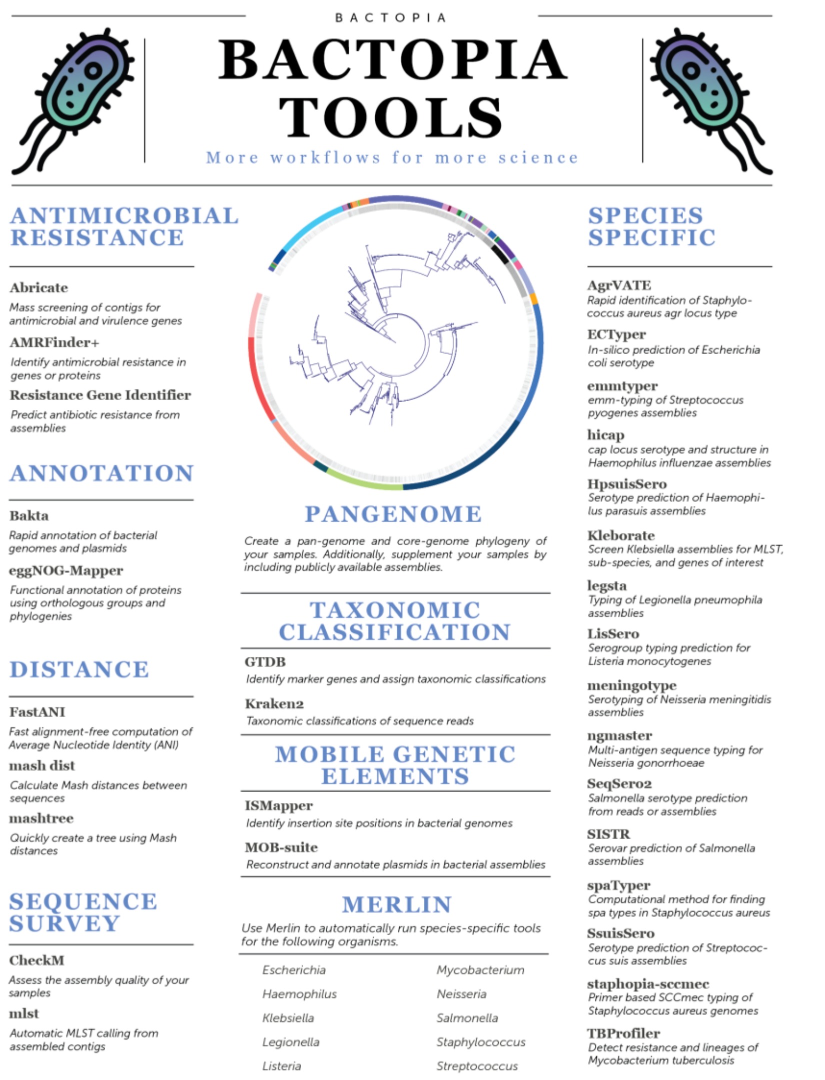
Easy comparative analysis of Bactopia outputs

Two Types:

- Single tool
  - *Kleborate, SeqSero2, TB Profiler*
- Multiple tools connected together
  - *pangenome: Prokka -> PIRATE -> IQ-Tree*

50+ Bactopia Tools are available

- Frame-worked for easy addition
- Ex. pangenome Bactopia Tool



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BACTOPIA TOOLS

More workflows for more science

ANTIMICROBIAL RESISTANCE

Abricate
Mass screening of contigs for antimicrobial and virulence genes

AMRFinder+
Identify antimicrobial resistance in genes or proteins

Resistance Gene Identifier
Predict antibiotic resistance from assemblies

ANNOTATION

Bakta
Rapid annotation of bacterial genomes and plasmids

eggNOG-Mapper
Functional annotation of proteins using orthologous groups and phylogenies

DISTANCE

FastANI
Fast alignment-free computation of Average Nucleotide Identity (ANI)

mash dist
Calculate Mash distances between sequences

mashtree
Quickly create a tree using Mash distances

SEQUENCE SURVEY

CheckM
Assess the assembly quality of your samples

mlst
Automatic MLST calling from assembled contigs

PANGENOME

Create a pan-genome and core-genome phylogeny of your samples. Additionally, supplement your samples by including publicly available assemblies.

TAXONOMIC CLASSIFICATION

GTDB
Identify marker genes and assign taxonomic classifications

Kraken2
Taxonomic classifications of sequence reads

MOBILE GENETIC ELEMENTS

ISMapper
Identify insertion site positions in bacterial genomes

MOB-suite
Reconstruct and annotate plasmids in bacterial assemblies

MERLIN

Use Merlin to automatically run species-specific tools for the following organisms.

Escherichia
Haemophilus
Klebsiella
Legionella
Listeria
Mycobacterium
Neisseria
Salmonella
Staphylococcus
Streptococcus

SPECIES SPECIFIC

AgrVATE
Rapid identification of Staphylococcus aureus agr locus type

ECTyper
In-silico prediction of Escherichia coli serotype

emmtyper
emm-typing of Streptococcus pyogenes assemblies

hicap
cap locus serotype and structure in Haemophilus influenzae assemblies

HpsuisSero
Serotype prediction of Haemophilus parasuis assemblies

Kleborate
Screen Klebsiella assemblies for MLST, sub-species, and genes of interest

legsta
Typing of Legionella pneumophila assemblies

LisSero
Serogroup typing prediction for Listeria monocytogenes

meningotype
Serotyping of Neisseria meningitidis assemblies

ngmaster
Multi-antigen sequence typing for Neisseria gonorrhoeae

SeqSero2
Salmonella serotype prediction from reads or assemblies

SISTR
Serovar prediction of Salmonella assemblies

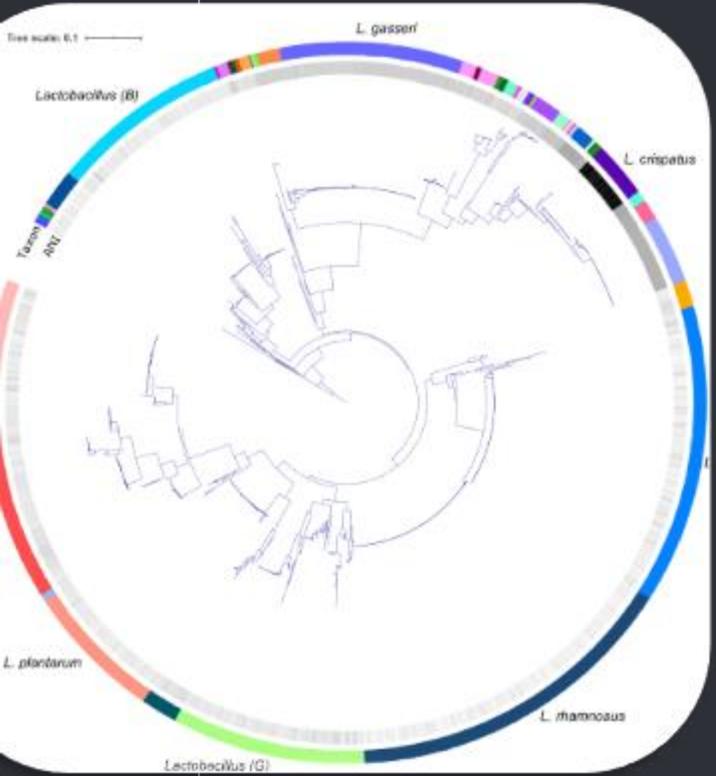
spaTyper
Computational method for finding spa types in Staphylococcus aureus

SsuisSero
Serotype prediction of Streptococcus suis assemblies

staphopia-sccmec
Primer based SCCmec typing of Staphylococcus aureus genomes

TBProfiler
Detect resistance and lineages of Mycobacterium tuberculosis

Bactopia Tools can simplify complex tasks



For example, you can quickly generate a phylogeny based on a core-genome, core-snps, 16S rRNA, or sketches.

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Design descisions

Let’s take a look at a few fundamental values behind Bactopia

“

Needed to set some ground rules to reduce the maintenance burden of Bactopia

Tools must be free and open-source

Tools must be available from Bioconda or Conda-Forge

Bactopia Tools must be available from nf-core/modules

> *These help ease the maintenance burden of Bactopia while unexpectedly facilitating contributions back to the community.*

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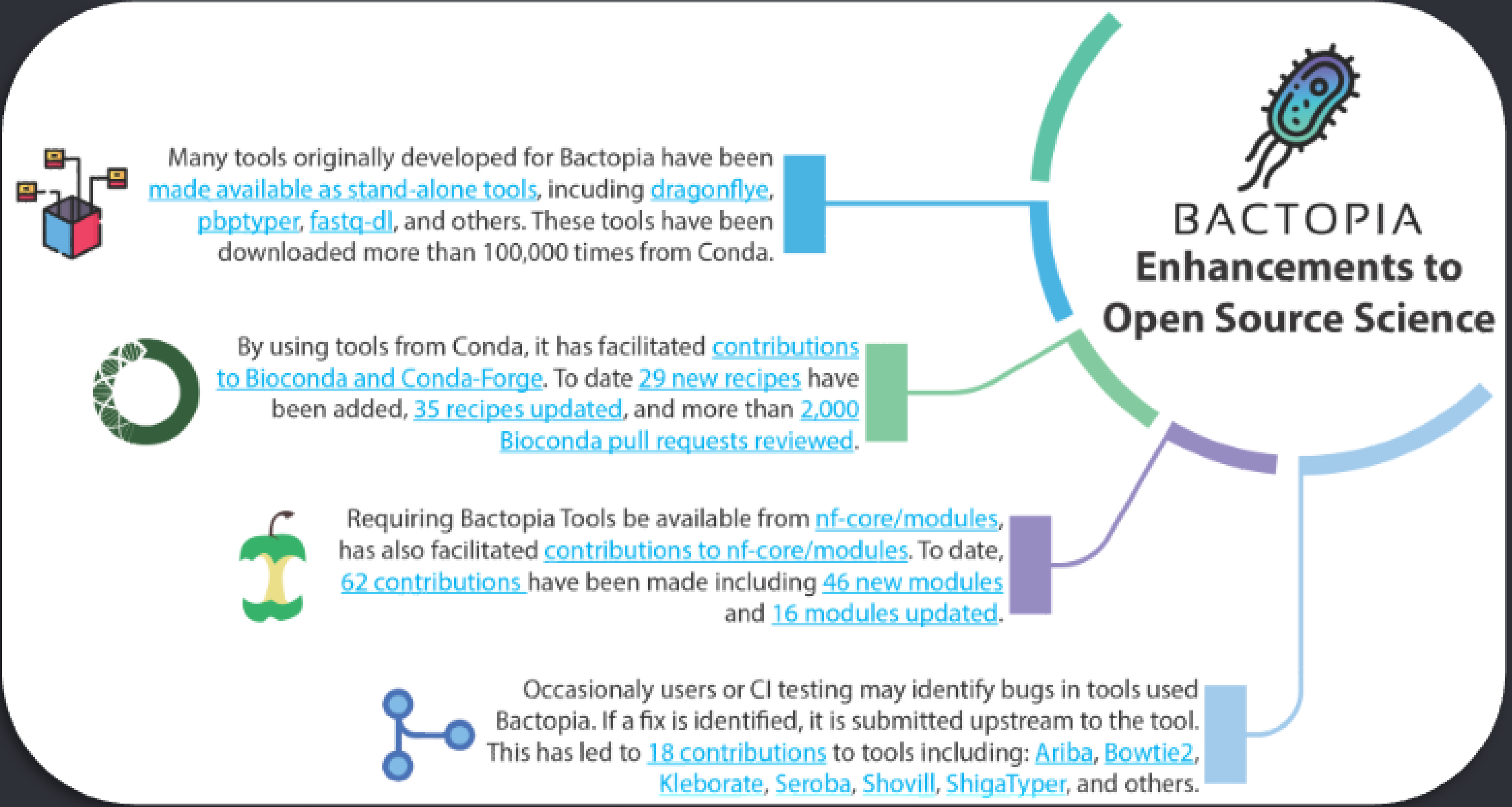
Enahncements to OSS

Let’s learn how Bactopia contributes back to the community

> *As a developer of a pipeline making use of hundreds of open-source tools, it is very import to me that I find ways to contribute back to the community*

Bactopia Enhancements to OSS



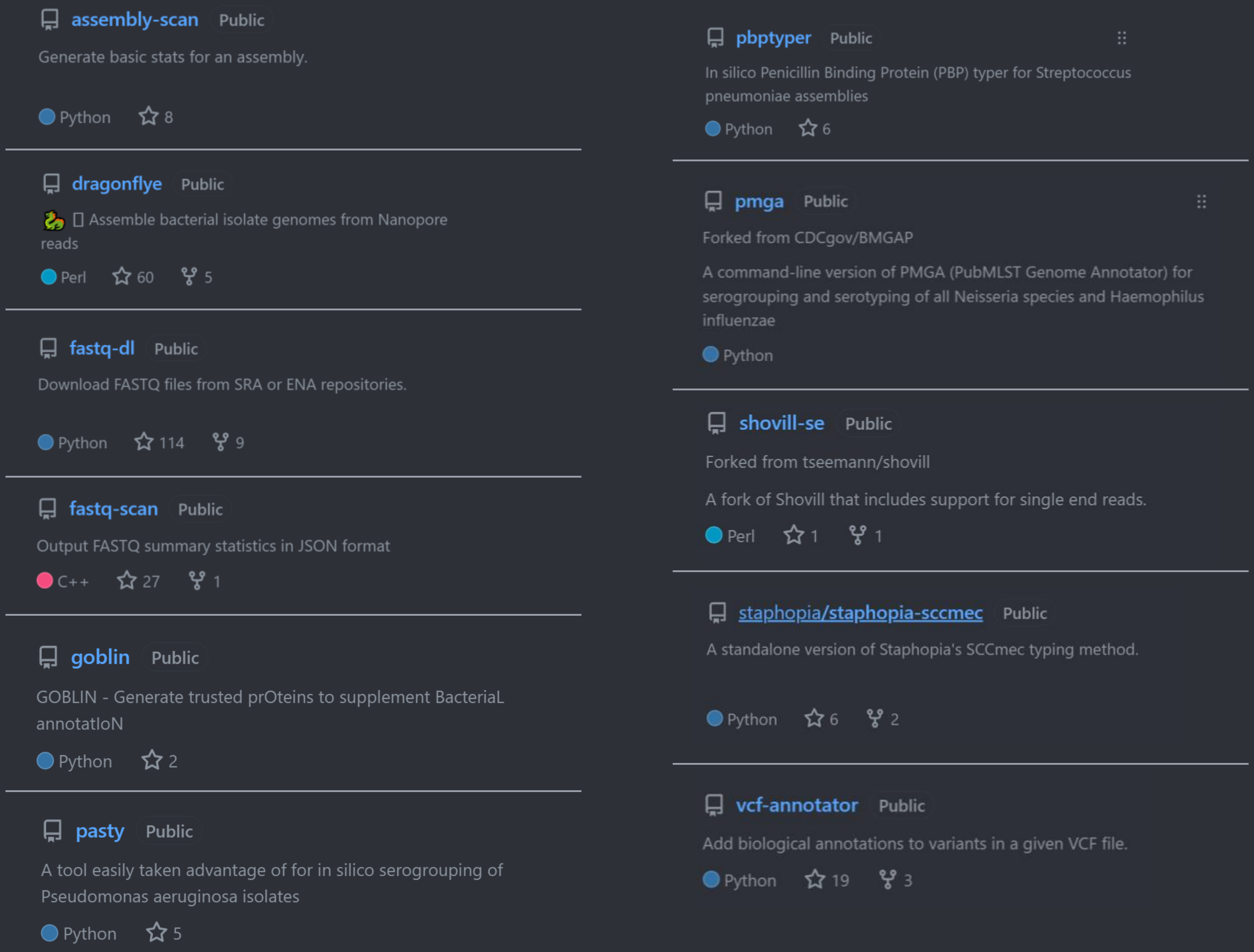
BACTOPIA
Enhancements to Open Source Science

Many tools originally developed for Bactopia have been made available as stand-alone tools, incuding dragonflye, pbptyper, fastq-dl, and others. These tools have been downloaded more than 100,000 times from Conda.

By using tools from Conda, it has facilitated contributions to Bioconda and Conda-Forge. To date 29 new recipes have been added, 35 recipes updated, and more than 2,000 Bioconda pull requests reviewed.

Requiring Bactopia Tools be available from nf-core/modules, has also facilitated contributions to nf-core/modules. To date, 62 contributions have been made including 46 new modules and 16 modules updated.

Occasionaly users or CI testing may identify bugs in tools used Bactopia. If a fix is identified, it is submitted upstream to the tool. This has led to 18 contributions to tools including: Ariba, Bowtie2, Kleborate, Seroba, Shovill, ShigaTyper, and others.



assembly-scan Public

Generate basic stats for an assembly.

● Python ☆ 8

dragonflye Public

🐉 ▯ Assemble bacterial isolate genomes from Nanopore reads

● Perl ☆ 60 ⑂ 5

fastq-dl Public

Download FASTQ files from SRA or ENA repositories.

● Python ☆ 114 ⑂ 9

fastq-scan Public

Output FASTQ summary statistics in JSON format

● C++ ☆ 27 ⑂ 1

goblin Public

GOBLIN - Generate trusted prOteins to supplement BacteriaL annotatIoN

● Python ☆ 2

pasty Public

A tool easily taken advantage of for in silico serogrouping of Pseudomonas aeruginosa isolates

● Python ☆ 5

pbptyper Public

In silico Penicillin Binding Protein (PBP) typer for Streptococcus pneumoniae assemblies

● Python ☆ 6

pmga Public

Forked from CDCgov/BMGAP

A command-line version of PMGA (PubMLST Genome Annotator) for serogrouping and serotyping of all Neisseria species and Haemophilus influenzae

● Python

shovill-se Public

Forked from tseemann/shovill

A fork of Shovill that includes support for single end reads.

● Perl ☆ 1 ⑂ 1

staphopia/staphopia-sccmec Public

A standalone version of Staphopia's SCCmec typing method.

● Python ☆ 6 ⑂ 2

vcf-annotator Public

Add biological annotations to variants in a given VCF file.

● Python ☆ 19 ⑂ 3

> *This separation simplifies the on-going maintenance of Bactopia and these tools.*

Through Bactopia contributions, I was invited to join the Bioconda Core Team, and the nf-core Modules Team

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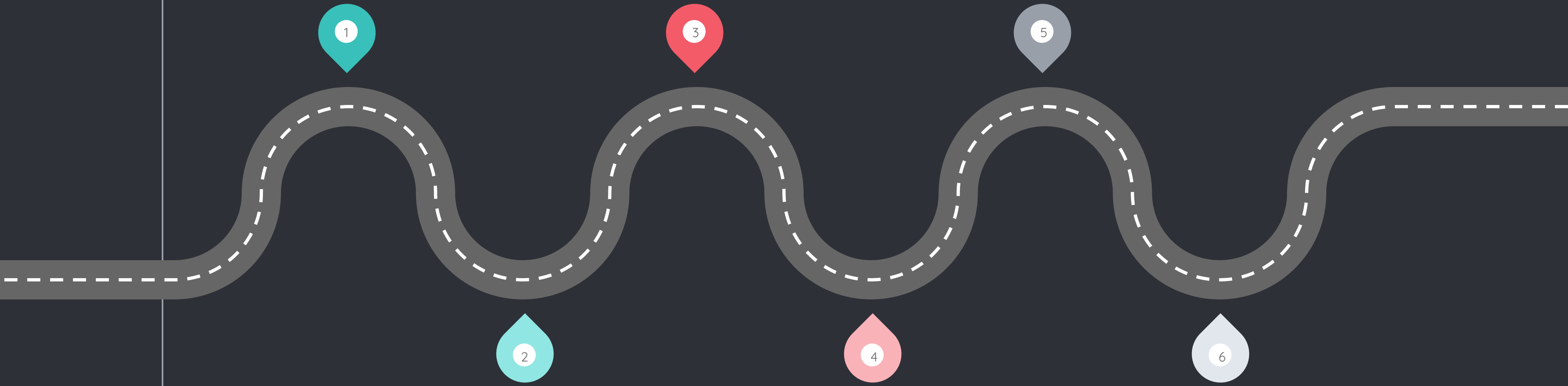
Future Directions

Let’s see what’s on the radar for Bactopia

Bactopia v3!

Major changes are inbound, and you’ll get a glimpse today!

Evolution of Bactopia



Circa 2011 – Development of Staphopia begins

Circa 2014 – Staphopia adopts a Python workflow manager called Ruffus

Circa 2017 – Staphopia adopts Nextflow, Conda, and Containerization

Circa 2019 – Staphopia is generalized to all bacteria, and thus Bactopia v1 is born

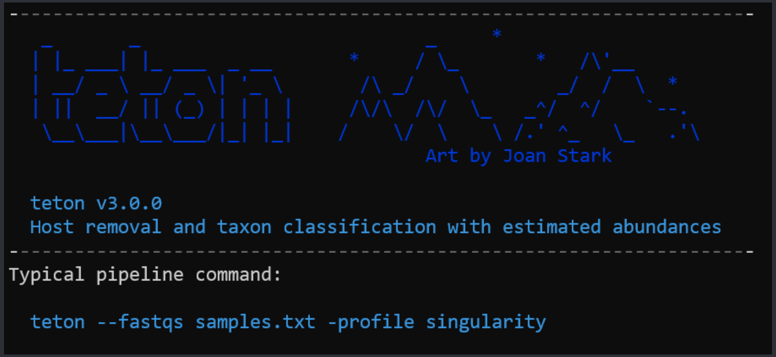
Circa 2021 – Bactopia v2 is released being rewritten in Nextflow DSL2

Today – Bactopia is nearing version 3. With many improvements geared towards on-going surveillance

Major changes inbound

- Bactopia Datasets no longer needed
- Multiple species per-run
- Improved directory structure
- A new Python package
  - https://github.com/bactopia/bactopia-py
- Additional workflows and Bactopia Tools
- Everything is a Bactopia Tool
- Many fixes and improvements
  - https://github.com/bactopia/bactopia/blob/dev/CHANGELOG.md

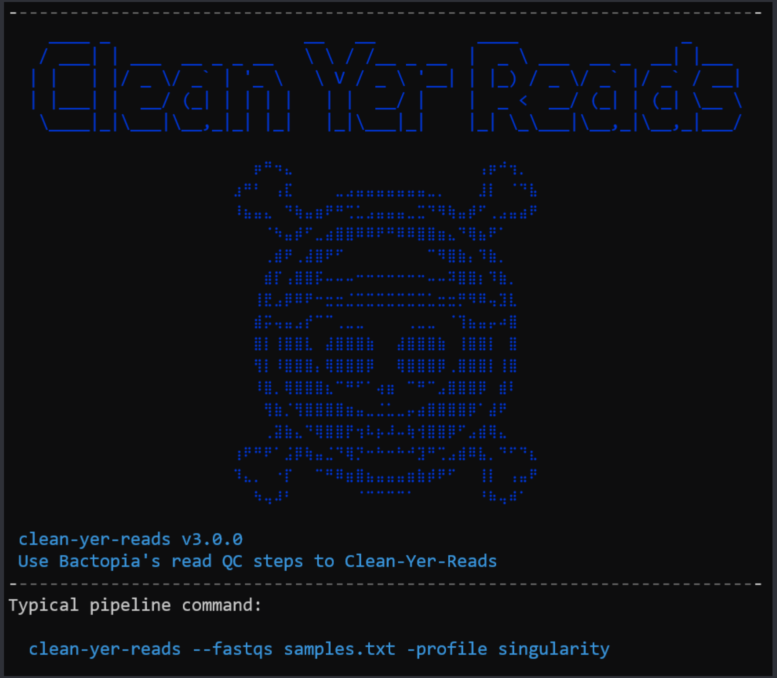
New Named Workflows



```
                                                  Art by Joan Stark

  teton v3.0.0
  Host removal and taxon classification with estimated abundances
----------------------------------------------------------------------
Typical pipeline command:

  teton --fastqs samples.txt -profile singularity
```



```
 clean-yer-reads v3.0.0
 Use Bactopia's read QC steps to Clean-Yer-Reads
----------------------------------------------------------------------
Typical pipeline command:

  clean-yer-reads --fastqs samples.txt -profile singularity
```

No significant changes required, just reshuffling Bactopia Tools

On the radar

- Customizable reports, starting with MultiQC
- Opened the door to metagenomics
- Full Nextflow Tower support
  - Terra.bio support depending on demand
- R Shiny app to view results interactively
- Additional features to the Python package
- Documentation updates
- Always more Bactopia Tools

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Wrap Up

Let’s see close this out and get the workshop started!

Bactopia is a robust pipeline for bacterial genome analysis

Accessible

Bactopia is open source and available from Conda, Docker and Singularity. Bactopia has been downloaded numerous times from users around the world.

Comprehensive

Bactopia is a start-to-finish pipeline which includes numerous tools and workflows commonly used for bacterial genome analysis.

Portable

With a simple profile change you can go from processing genomes on your laptop to an HPC system or any of the major cloud providers (AWS, GCP, Azure).

Reproducible

Bactopia was developed following nf-core best practices which ensures a robust pipeline with strict version control and an extensive audit trail.

Resilient

More than 100 tests, testing 10,000+ variables, assist in identifying potential bugs and downstream changes, before users are affected.

Scalable

Bactopia allows you to easily scale from a few genomes to thousands of genomes. For example, processing 67,000 genomes in 5 days on AWS.

Who and where is Bactopia being used?

849,200

Docker container pulls – that's a lot!

47,500

Conda environments built

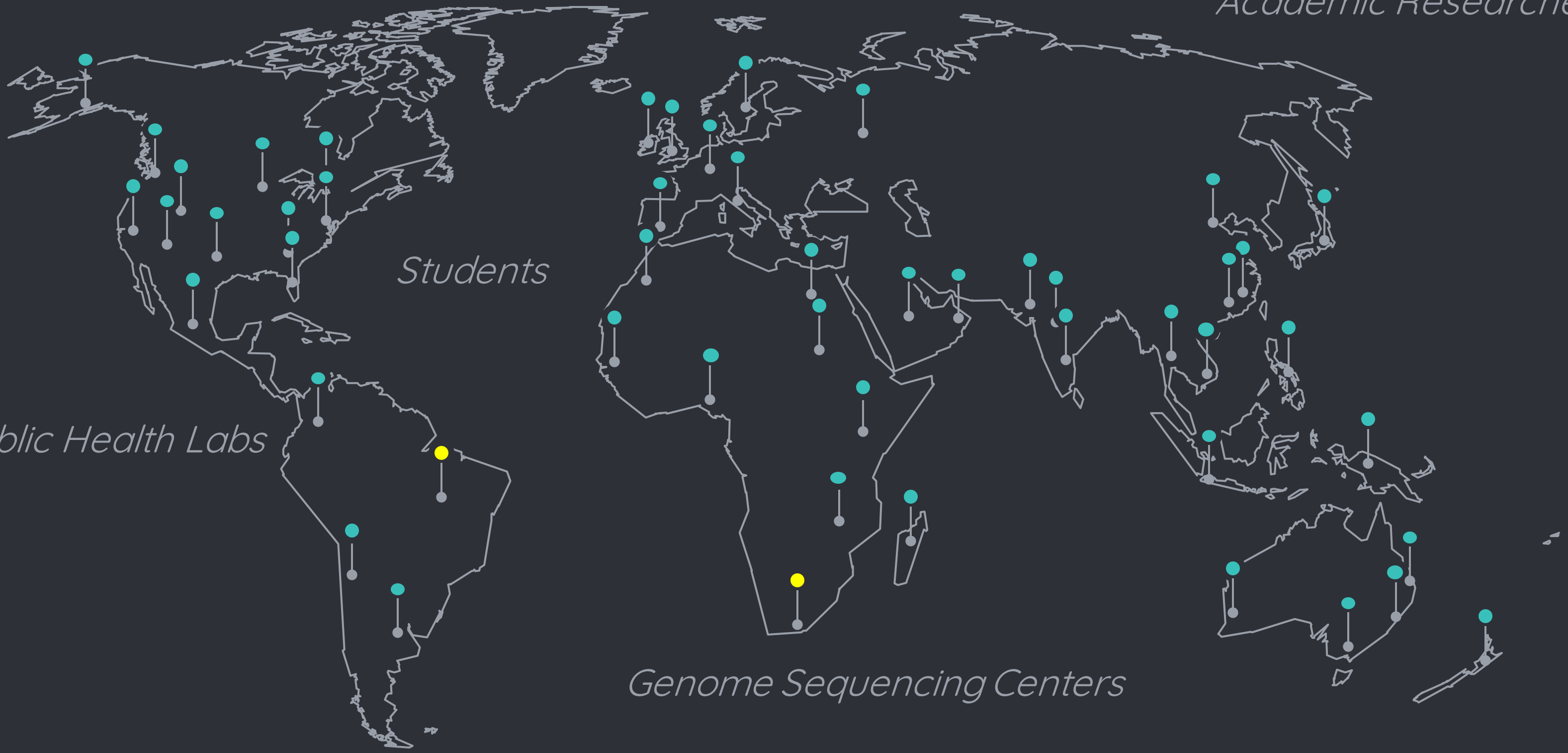
34,500 visitors

Many users around the world visiting the docs

229

GitHub stars!

Bactopia Users Across the Globe



Academic Researchers

Students

Public Health Labs

Genome Sequencing Centers

Many of these dots are in response to direct communications

Acknowledgements

All the developers of open-source software used by Bactopia, and the many users regularly providing feedback and suggestions

Wyoming Public Health
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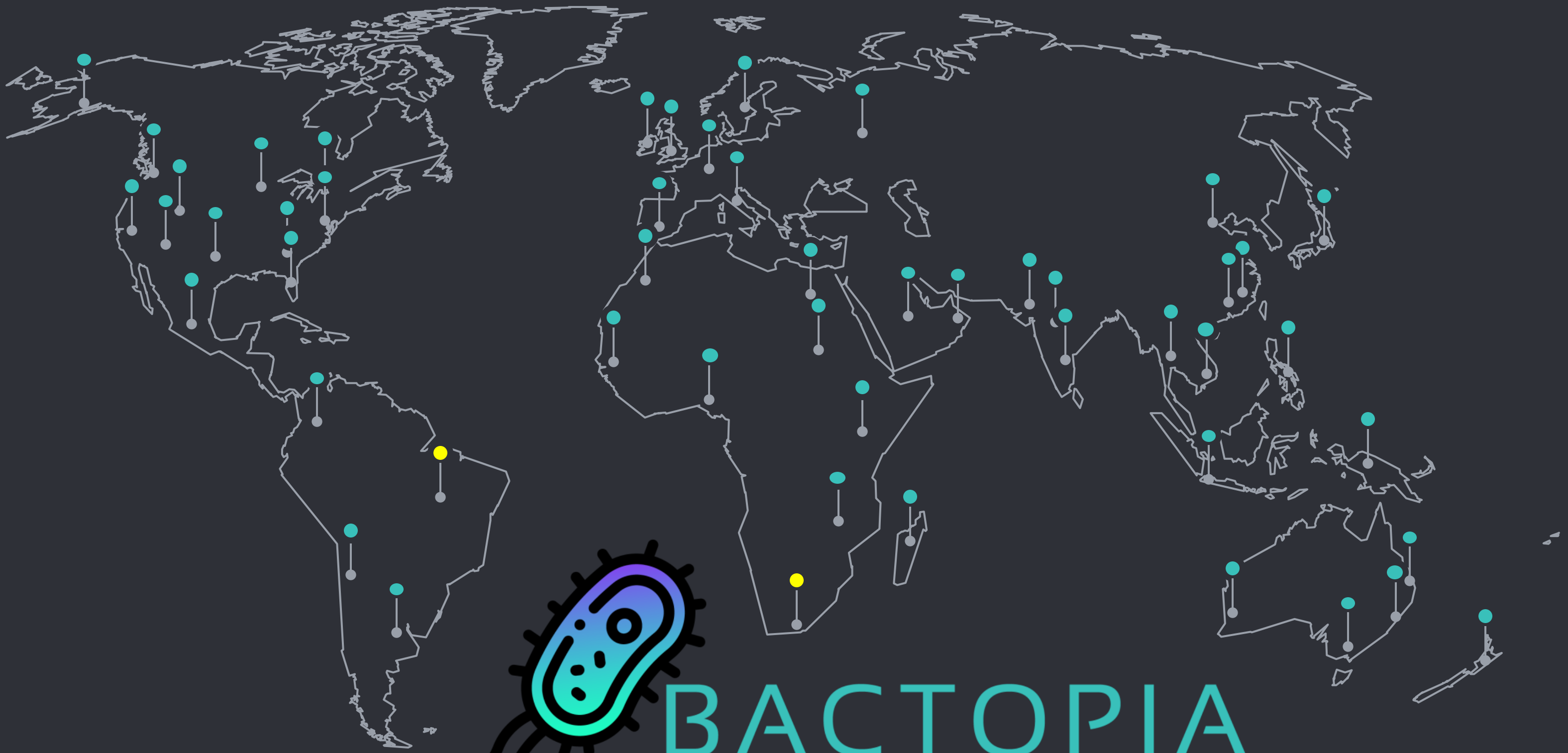
BIOCONDA®

nextflow

nf-core

Presentation template by SlidesCarnival

Any Questions?



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Bactopia Workshop

Bactopia Tools Workshop