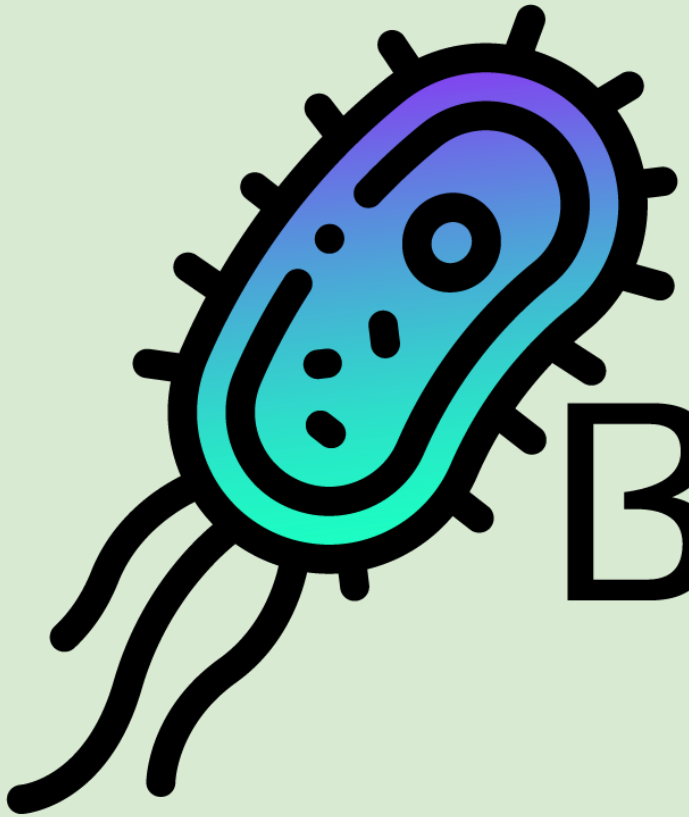


BACTOPIA

Using Bactopia for the complete analysis of bacterial genomes

April 28th, 2022

Robert A. Petit III, PhD

@rpetit3 @rpetit3

Theiagen Genomics

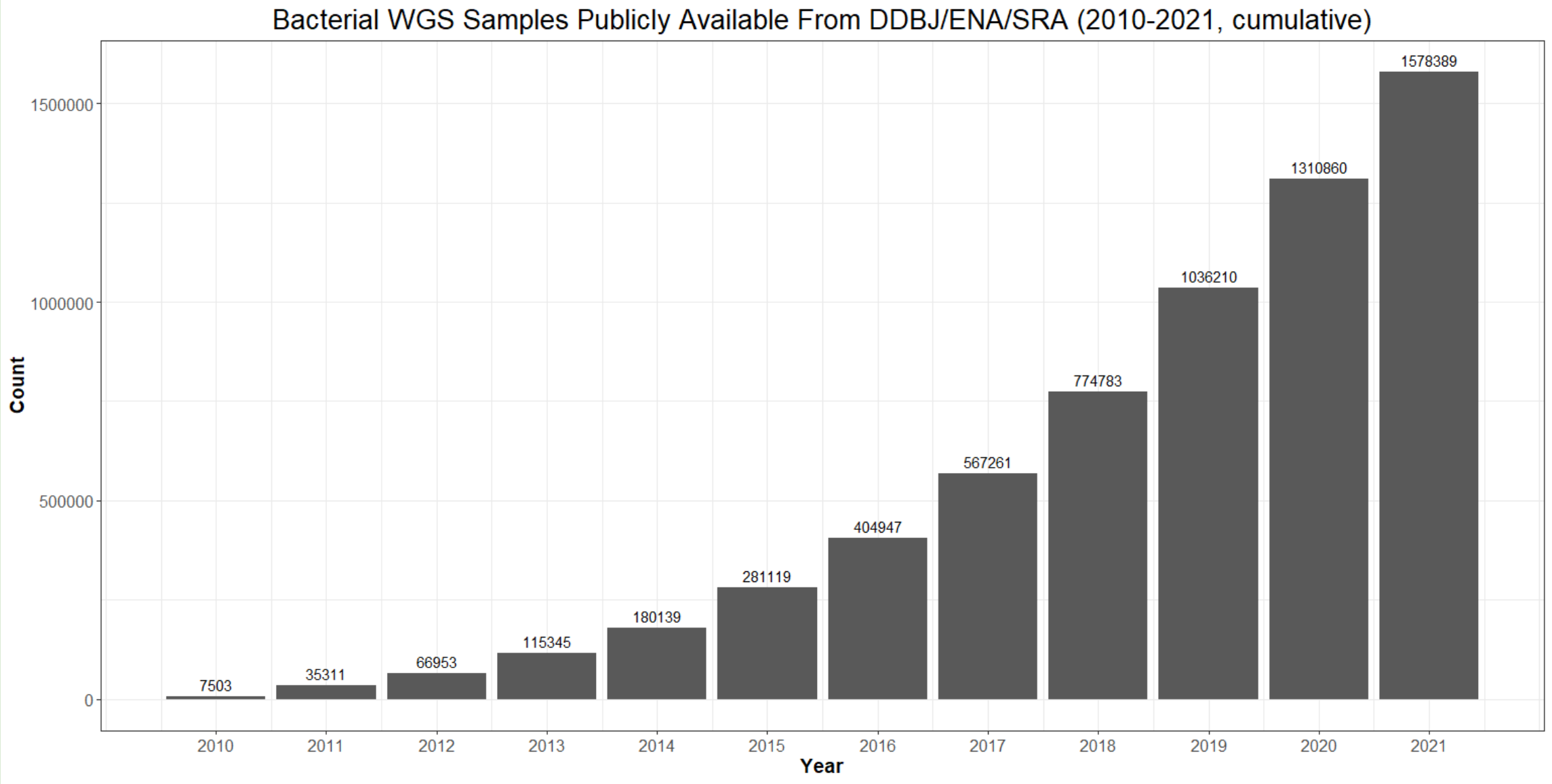
Overview

- Background and Motivations
- Learn about Bactopia
- Use Case: Using Bactopia to describe public Lactobacillus genomes
- Future Directions

Background and Motivations

Bacterial genomics is a rapidly evolving field

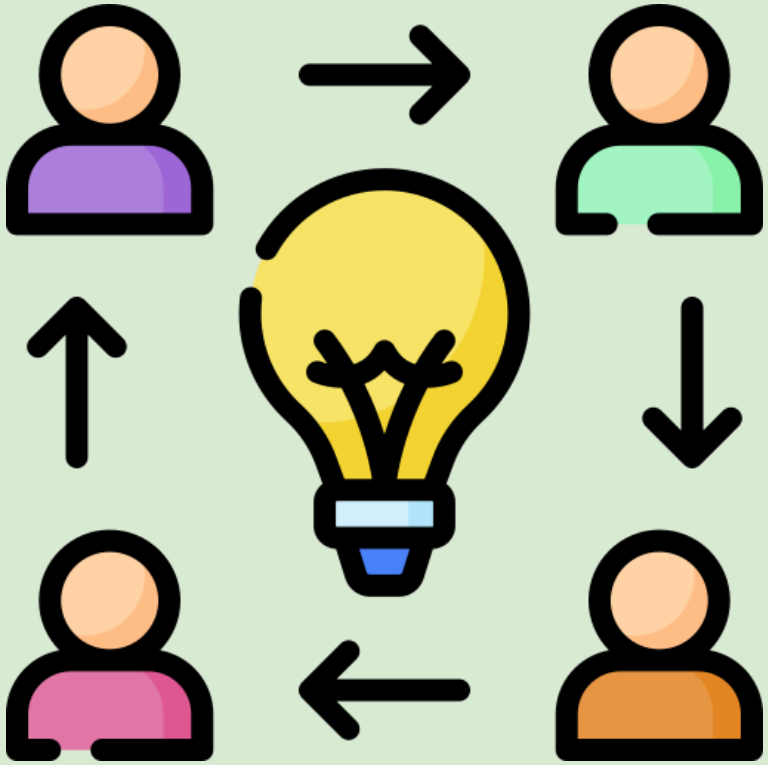
Rapid growth of bacterial WGS in the last 10 years



Rapid growth in the bioinformatics as well

Conda, containerization, workflow managers, etc...

BIOCONDA® for all your bioinformatic tools



- Makes bioinformatics accessible
  - Easy installs, dependency handling
- Downstream containerization
  - Docker – Biocontainers
  - Singularity Images - Galaxy Project
- Truly a community driven repository
  - More than 1,300 people have contributed
- Currently ~4,000 recipes are available

Workflow managers make bioinformatics manageable

- Manages the execution of pipelines
  - Linking inputs/outputs of bioinformatic tools
  - Queuing jobs locally, on clusters, or the cloud
  - Logging, errors, audit trails
- Promote reproducible and reusable science
- Common workflow languages:
  - Nextflow, WDL, and Snakemake
- Pick one that works for you

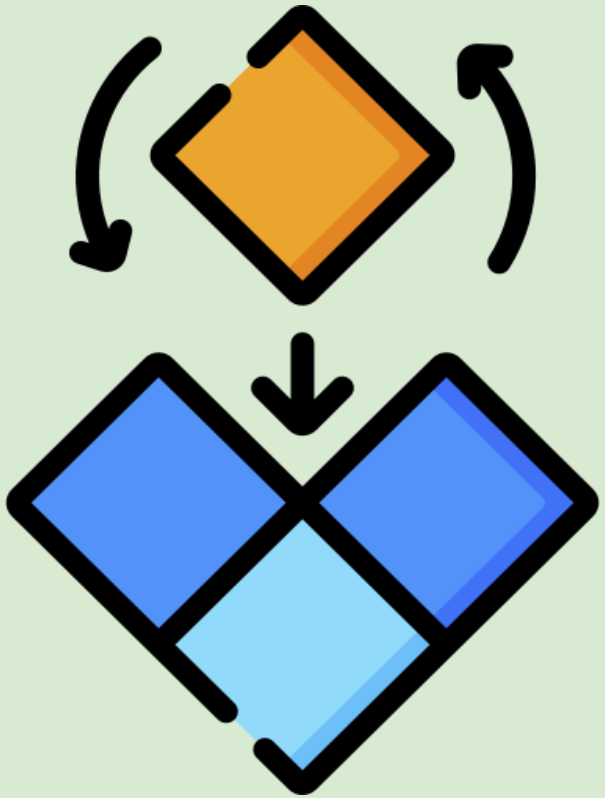
nextflow

```
nextflow.enable.dsl=2


process sayHello {
  input:
    val cheers
  output:
    stdout

  """
  echo $cheers
  """
}

workflow {
  channel.of('Ciao','Hello','Hola') | sayHello | view
}
```

- A popular workflow manager in Bioinformatics
- Enables scalable and reproducible pipelines
- Supports Conda, Docker, and Singularity
- Seamlessly move between local resources, HPC, and major cloud providers
- Regularly solicits user feedback to guide future developments

nextflow DSL2

- Major evolution in the Nextflow language
- Introduced true modularization in Nextflow workflows
  - Modules – A ***reusable*** Nextflow script with a process definition
  - Subworkflows – Multiple modules linked together
- Modules are portable and easily shared between workflows
- Data channels can be used more than once



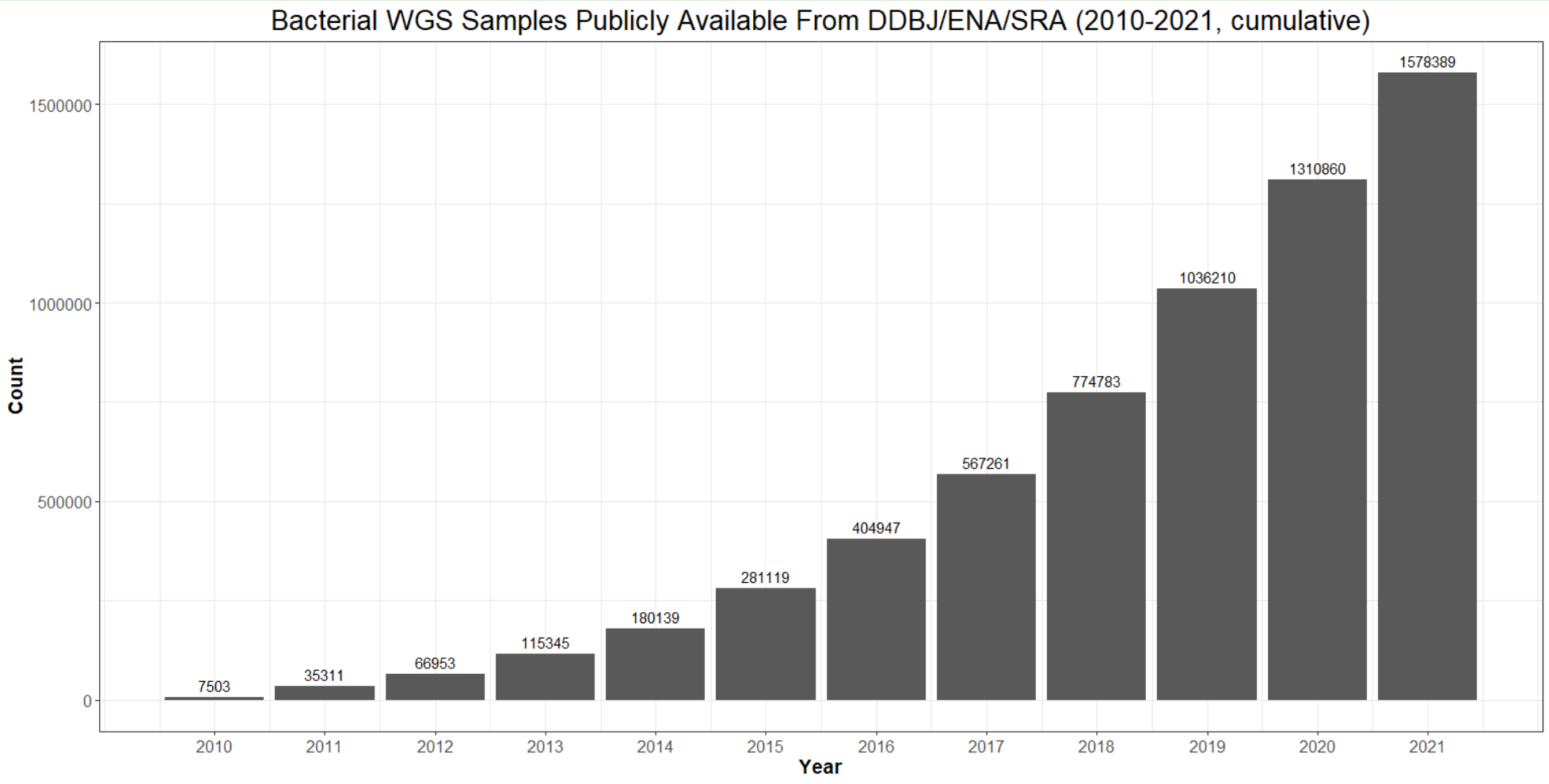
nf-core pushing Nextflow to the limits

- Community effort to collect curated Nextflow pipelines
  - 2400 Slack users, 1000+ GitHub contributors
- Includes 60+ hi-quality bioinformatic pipelines
  - rnaseq, mag, bactmap, many more
- nf-core/modules has 200+ DSL2 modules available
- Standardized guidelines for developers
- Thorough review process produces robust pipelines

"Bring your own workflow" Platforms

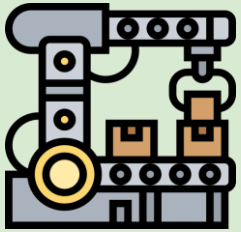
- Freely available web-platforms for the execution of bioinformatic pipelines
- No command-line knowledge required, allowing users to do more science
- Examples:
  - Nextflow Tower from Seqera Labs
    - Supports workflows written in Nextflow
    - Platform agnostic and supports many providers
      - HPC, Google Cloud, Microsoft Azure, Amazon Web Services
    - Community showcase of curated pipelines
  - Terra from the Broad Institute
    - Supports workflows written in WDL
    - Limited to Google Cloud Platform
      - Microsoft Azure support in the works
    - Import workflows from Dockstore
  - CGC from Seven Bridges
    - Supports workflows written in CWL, Nextflow and WDL
    - Limited to Amazon Web Services

With the bioinformatic advances, it's hard to ignore public WGS



How can we make use of all this public WGS data?

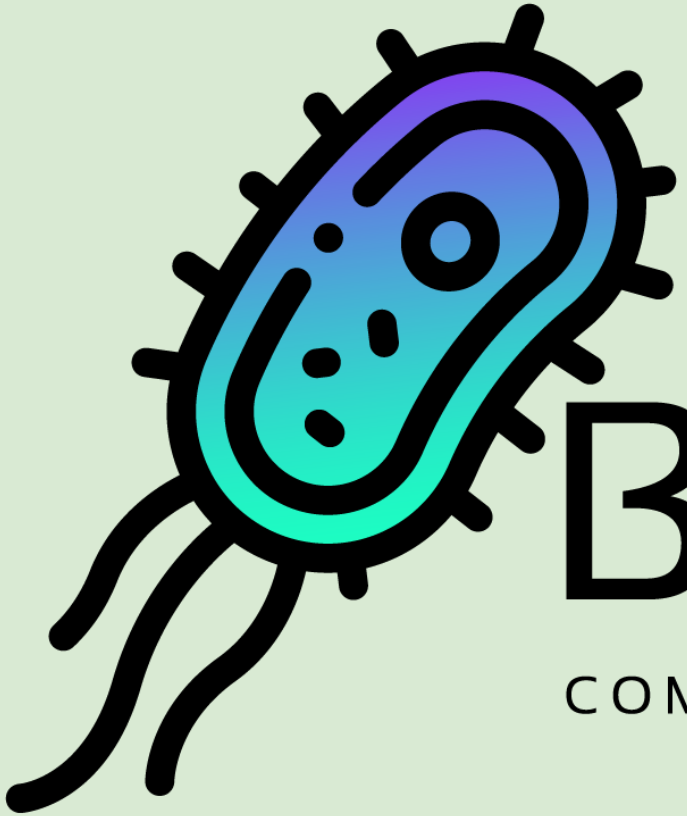
To take advantage of public data we need:



- Ensure each sample is processed the same way
  - Allows comparison of your data with public data

- Scalable pipeline to process 1 or 1,000s of samples
  - Should also be easy to use, reproducible, and portable

- Account for poor quality data
  - Example: FASTQs missing pairs, mislabeled species



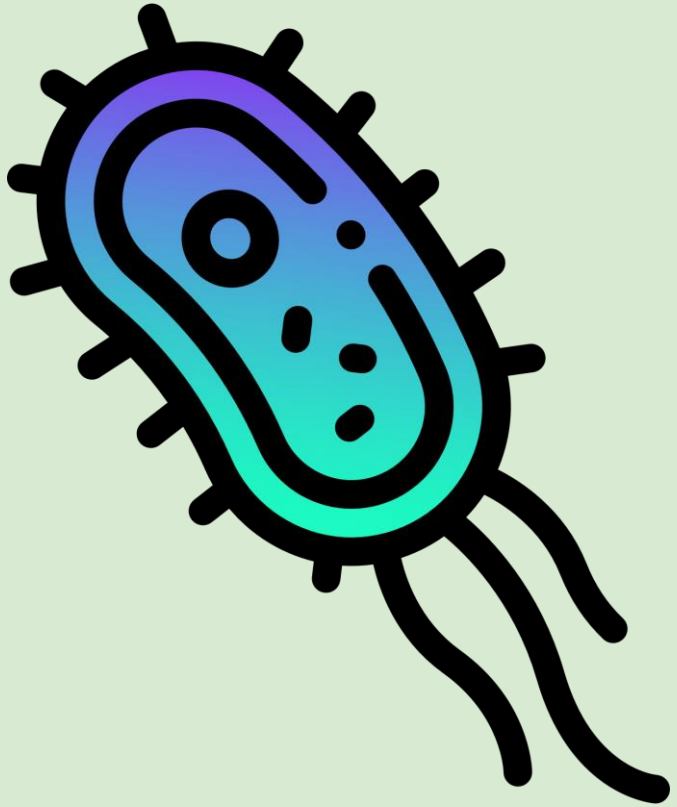
BACTOPIA

COMPLETE ANALYSIS OF BACTERIAL GENOMES

Bactopia is a scalable, reproducible, and portable all-in-one pipeline.

A few Bactopia highlights

- Supports Illumina and Nanopore reads
  - Local or from public databases
- Includes more than 130 bioinformatic tools
- 30+ Bactopia Tools provide more workflows for more science
- Extensively tested with 100+ tests for 10,000+ output files
- Available on Bioconda, Docker, and Singularity
- Well documented at bactopia.github.io



Bactopia design principles

Bactopia requires tools be available from Bioconda

- Easy to install, downstream Docker and Singularity containers

All modules used by Bactopia Tools must be on nf-core/modules

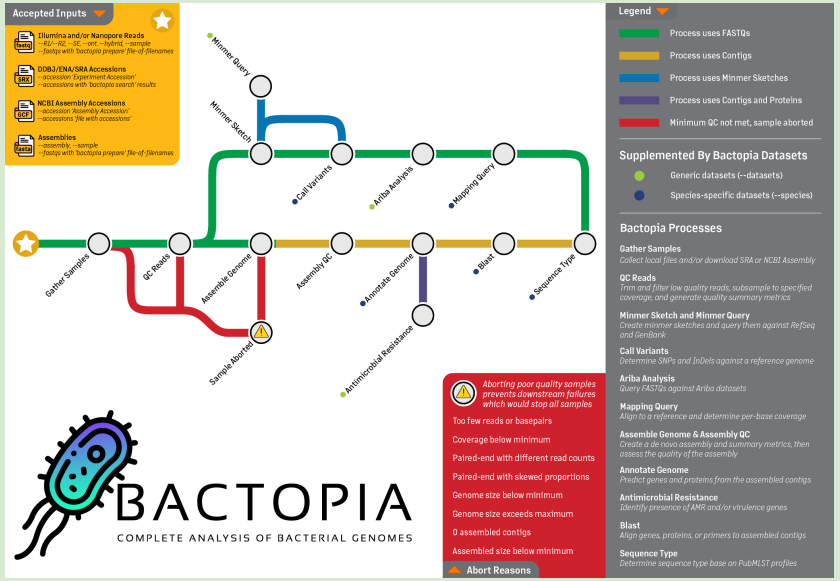
- Developers can use these modules in their own workflows

Bactopia should be flexible and easily adapted to fit user needs

- Nextflow DSL2 has made this much easier

Three Sides of Bactopia

Bactopia Helpers



Bactopia

Bactopia Tools

Bactopia Helpers, help get started using Bactopia

- bactopia citations
  - Print citations for all datasets, tools and Bactopia
- bactopia datasets
  - Download and setup useful datasets for Bactopia
- bactopia download
  - Build Conda, Docker or Singularity environments for all steps in bactopia
- bactopia prepare
  - Create a file of filenames for analysis
- bactopia search
  - Generate a list of SRA accessions for analysis

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

BACTOPIA

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 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

Ariba Analysis
Query FASTQs against Ariba datasets

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

- Allow easy comparative analysis of Bactopia outputs
- Two Types:
  - Modules: single tool (Kleborate, TB Profiler)
  - Subworkflows: multiple tools connected together
    - pangenome: Prokka -> PIRATE -> IQ-Tree
- 30+ Bactopia Tools are available
  - Frame-worked for easy addition
  - Simple command to create new Bactopia Tool

BACTOPIA

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)

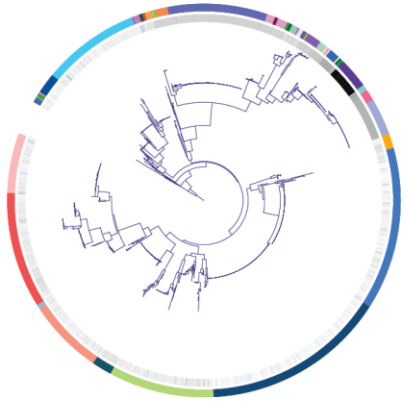
mash dist
Calculate Mash distances between sequences

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	*Mycobacterium*
Haemophilus	*Neisseria*
Klebsiella	*Salmonella*
Legionella	*Staphylococcus*
Listeria	*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

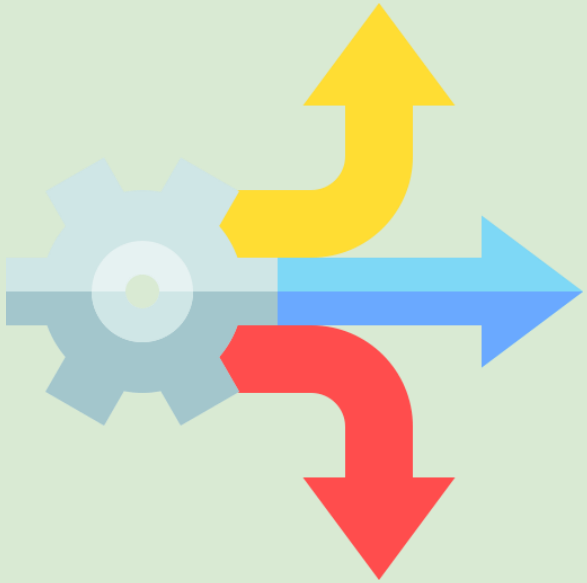
TBProfiler
Detect resistance and lineages of Mycobacterium tuberculosis

Because of DSL2, every Bactopia Tool can be reused!

Demonstration of Bactopia Tool reusability

- There are 15+ species-specific Bactopia Tools
  - Sometimes the species is known
  - Sometimes the species is unknown

Can we automatically execute these species-specific tools?



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TBProfiler
Detect resistance and lineages of Mycobacterium tuberculosis

Merlin - **M**inm**ER** assisted species-specific bactopia tool se**L**ect**I**o**N**

- Mash distances select species-specific tools
  - Re-uses the RefSeq Mash Sketch downloaded by Bactopia Datasets

- Currently includes members of 10+ genera

MERLIN

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

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Klebsiella	*Salmonella*
Legionella	*Staphylococcus*
Listeria	*Streptococcus*

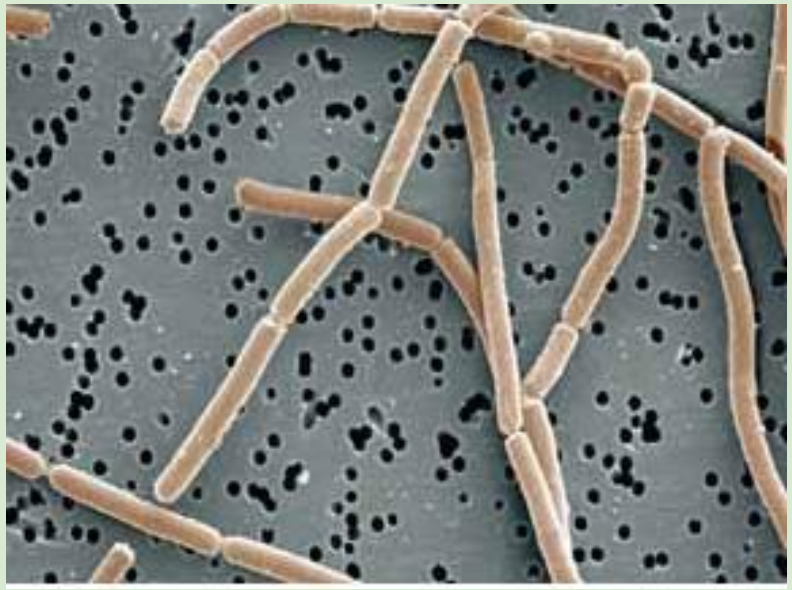
- Highlights module reuse and condition-based execution

Describing public Lactobacillus genomes

In just a few commands using Bactopia

Use Case: Investigating Lactobacillus

- Gram-positive, rod-shaped bacterium
- Common in human and animal microbiota
  - 40+ species adapted to microbiome hosts
  - Gastrointestinal and vaginal
  - Inhibits growth of other bacteria
- Economic uses include food production and probiotics
- We can use Bactopia to analyze Lactobacillus genomes in just a few commands



Lactobacillus bulgaricus
From Utah State University

Bactopia Helpers to get things setup

- Build public datasets with "*bactopia datasets*"
  - Downloads general and Lactobacillus-specific datasets
  - Ex. `bactopia datasets –species "lactobacillus"`
- "*bactopia search*" to identify publicly available WGS of Lactobacillus
  - Creates a list of accessions for Bactopia to process
  - Ex. `bactopia search 1578`

Use Case: Process Lactobacillus WGS

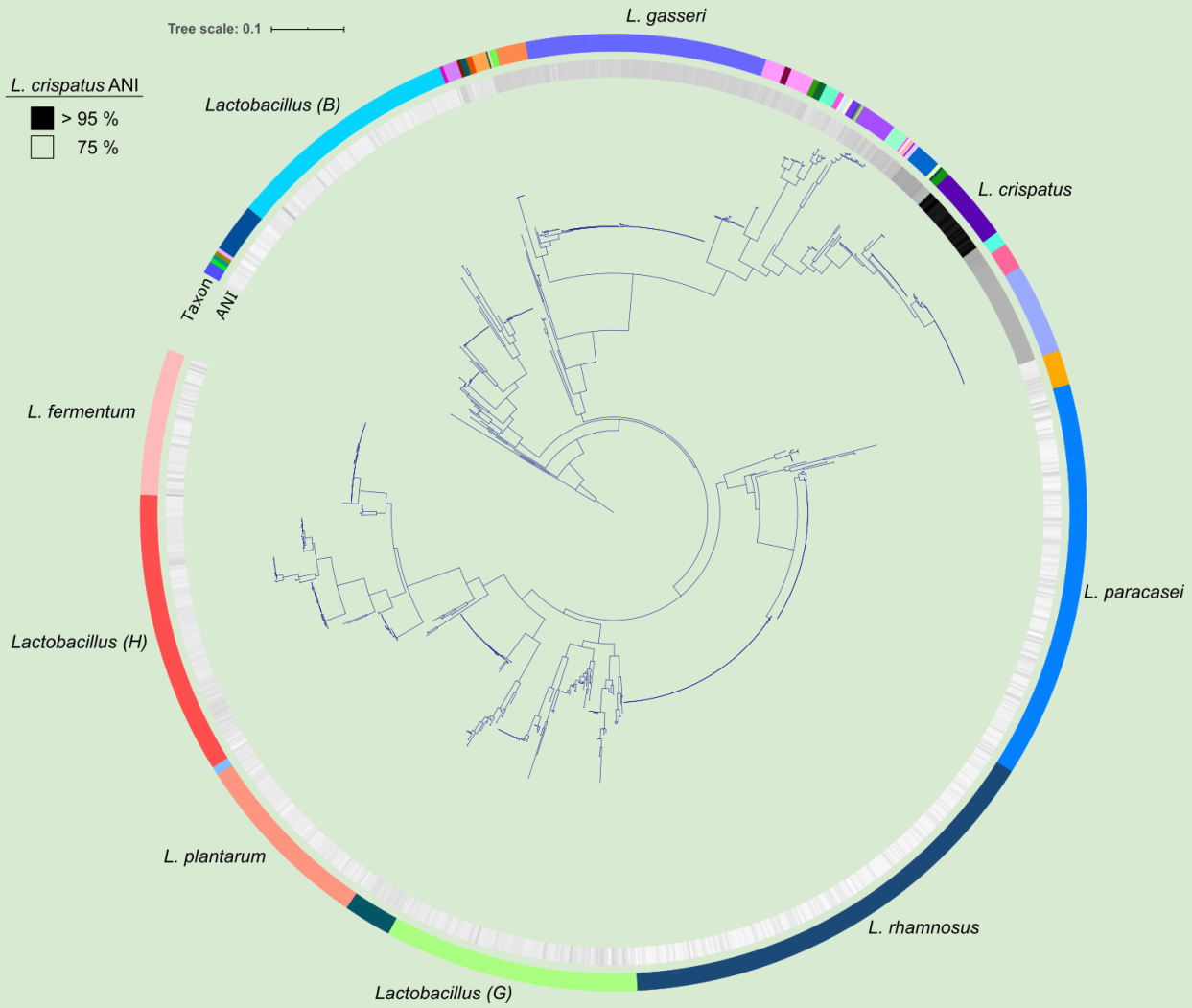
- Processed 1,664 genomes with Bactopia
  - Bactopia handles downloading from SRA/ENA

- Most of the samples were good quality

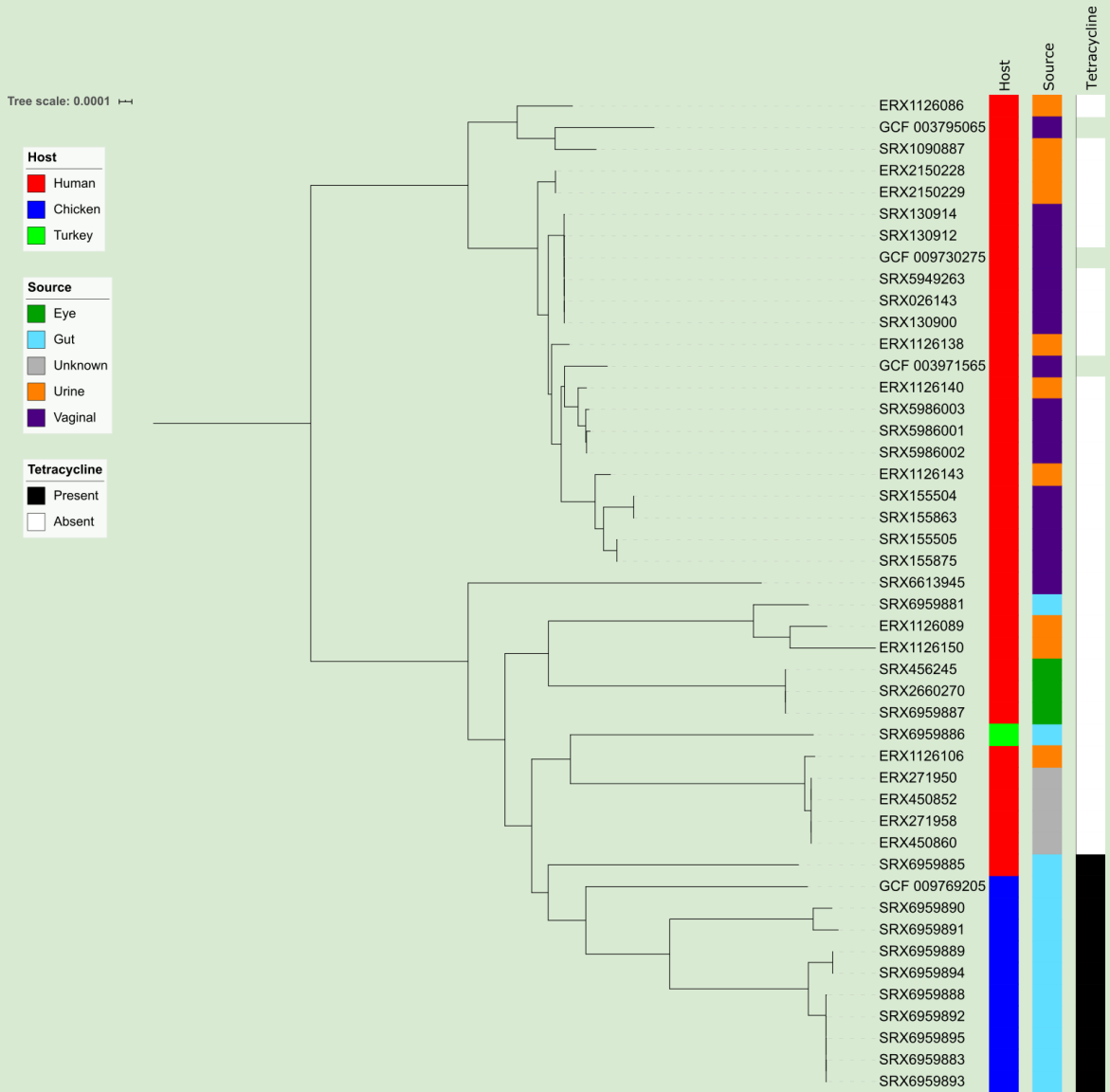
Quality rank	No. of samples	Original coverage	Post-Bactopia coverage	Per-read quality score	Read length (bp)	Contig count
Gold	967	213×	100×	Q35	100	52
Silver	386	160×	100×	Q35	100	110
Bronze	205	102×	100×	Q34	100	90
Exclude	48	26×	22×	Q34	100	706
Unprocessed	58					

Use Case: Lactobacillus 16S Tree

- phyloflash and gtdb Bactopia Tools
  - PhyloFlash for 16S construction
  - GTDB for taxonomic classification
- 5 species represented 45% of the samples
- 58 samples were not Lactobacillus



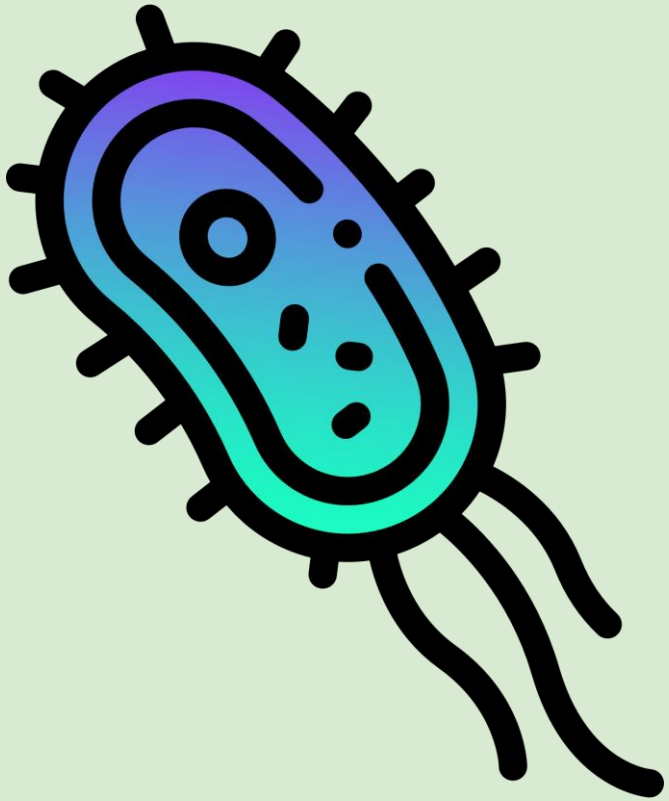
Use Case: Closer Look at *L. crispatus*



- fastani and pangenome Bactopia Tools
  - FastANI to identify *L. crispatus*
  - Roary to create core-genome alignment
  - IQ-TREE for core-genome phylogeny
- 38 *L. crispatus* samples clustered into two groups
  - Human vaginal samples
  - Chicken, turkey, human gut

Use Case: A few Bactopia commands from start to finish

- With just a few commands we analyzed 1,600 public WGS genomes
  - Built general and Lactobacillus specific datasets
    - *bactopia datasets*
  - Identified all publicly available Lactobacillus genomes
    - *bactopia search*
  - Processed all available Lactobacillus genomes
    - *Bactopia*
  - Taxonomic classification and core-genome phylogeny
    - *Bactopia Tools*
    - PhyloFlash, GTDB, FastANI, Roary, IQ-TREE

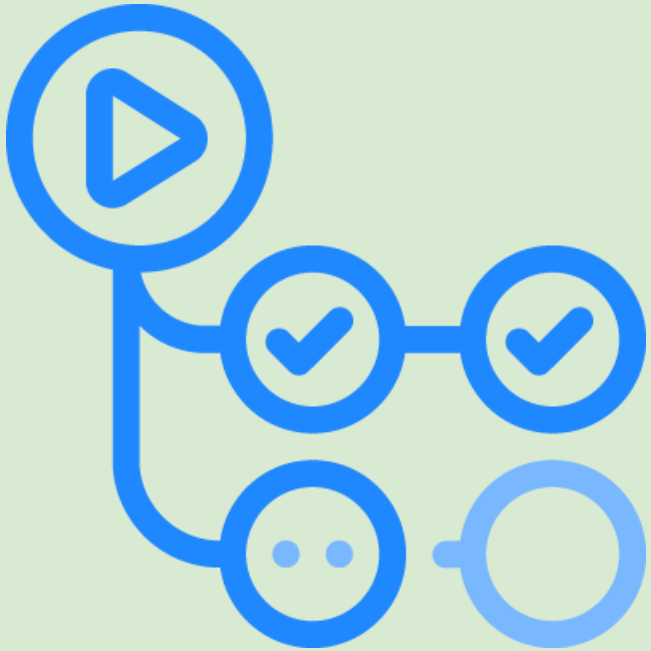


Additional Bactopia highlights

Bactopia as an introduction to bacterial genomics

- More than 130 bioinformatic tools are used by Bactopia
  - Description, citation, and code links are provided for each tool
- Bactopia is easily installed through Bioconda
- Bactopia is well documented
  - Detailed pipeline overview
  - Tutorial to get started
  - Output file descriptions

Tests keep the wheels turning



- Every step is tested in Bactopia using real data
  - Usages, workflows, subworkflows, modules
- More than 10,000 output files are tested
  - MD5sums match, contains strings, file exists
- Testing conducted by pytest
  - Adapted from nf-core/modules
- All automated through GitHub Actions

By using Nextflow, Bactopia is platform independent

- Execute on a laptop of the cloud, with a simple parameter change
- Available on:
  - Bioconda, Docker, Singularity
  - Google Cloud Platform
  - Amazon Web Services
  - Microsoft Azure
  - HPC
- Executable from:
  - Nextflow Tower
  - Terra.bio

Bactopia contributes back to the community

Bactopia requires tools be available from Bioconda

- Submitted 20 recipes, reviewed 1300+ Bioconda pull requests

All Bactopia Tools must be available from nf-core/modules

- Submitted 30+ modules to nf-core/modules

Multiple PRs submitted, and issues resolved

- Bactopia user finds bug in “X” tool, I attempt to fix and submit a PR
- Bowtie2, Prokka, Shovill, PIRATE, Ariba, ISMapper, PhyloFlash, etc….

Bactopia has spawned stand-alone tools

assembly-scan 4 5,483

Generate basic stats for an assembly

summary fasta assembly

dragonflye 44 3,725

Assemble bacterial isolate genomes from Nanopore reads

dragonflye bioconda

fastq-dl 30 4,791

Download FASTQ files from SRA or ENA repositories.

fastq sra ena

fastq-scan 23 14,357

Output FASTQ summary statistics in JSON format

summary fastq

shovill-se 0 1,726

A fork of Shovill that includes support for single end reads

assembly single-end

vcf-annotator 15 4,852

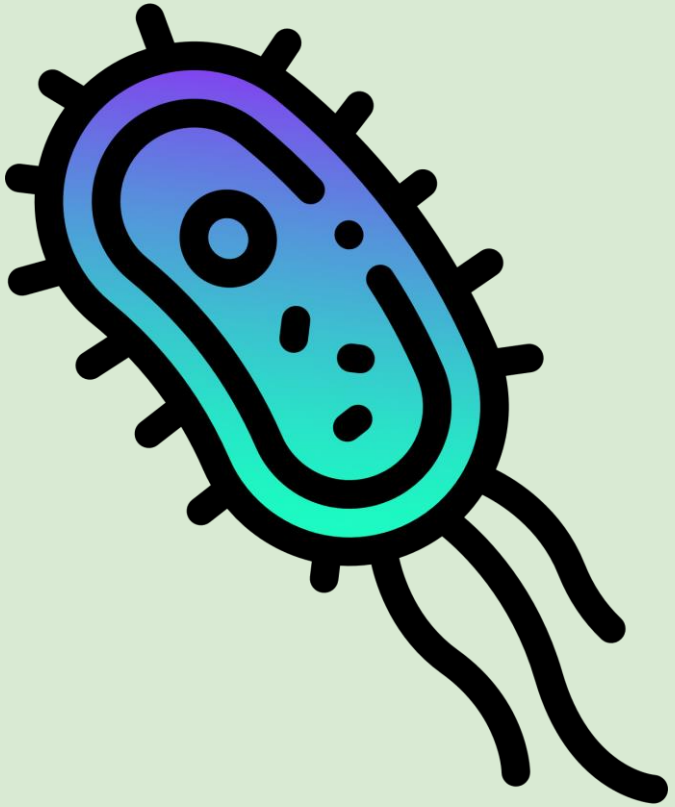
Add biological annotations to variants in a given VCF file.

annotation vcf genbank variant

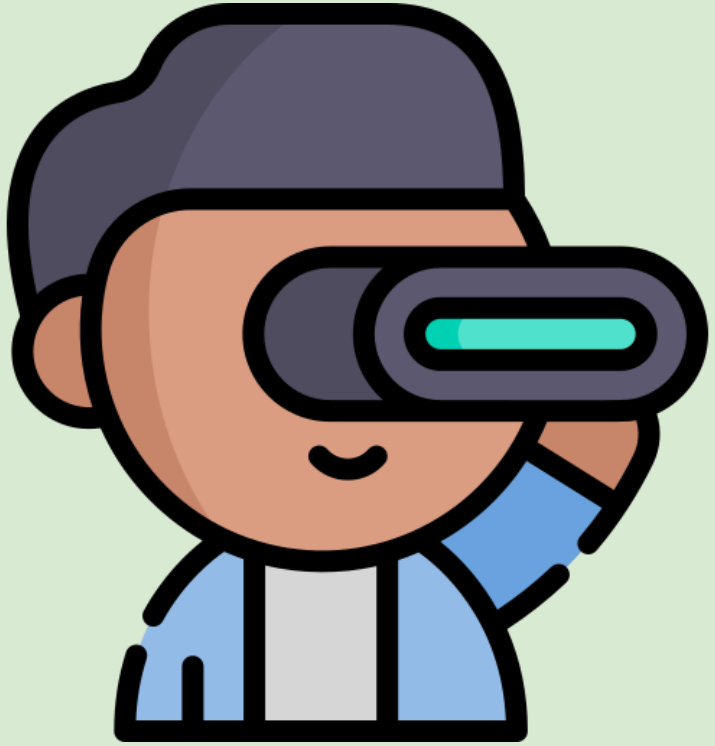
Conclusions

Bactopia summary

- Supports Illumina and Nanopore reads
- Includes more than 130 bioinformatic tools
- 30+ Bactopia Tools provide more workflows for more science
- Extensively tested with 100+ tests for 10,000+ output files
- Available on Bioconda, Docker, and Singularity
- Well documented at bactopia.github.io



Future directions for Bactopia



- Bactopia still has a lot of room to grow
- Future directions
  - Improve the result reporting
  - Additional Bactopia Tools
  - Expansion of the documentation
- Always open to contributions and user feedback

Acknowledgements

The developers of open-source tools that make their tools freely available to the community

- Tim Read and the EMERGENT Group
- Davi Marcon, Abhinav Sharma, and nf-core
- Wyoming Public Health Laboratory
  - Taylor Fearing
  - Jim Mildenberger
  - Chayse Rowley
- Theiagen Genomics
- The many users of Bactopia providing feedback and helping guide future developments