



In-silico performance of a targeted enriched metagenomics approach to infer *Mycoplasma bovis* strains

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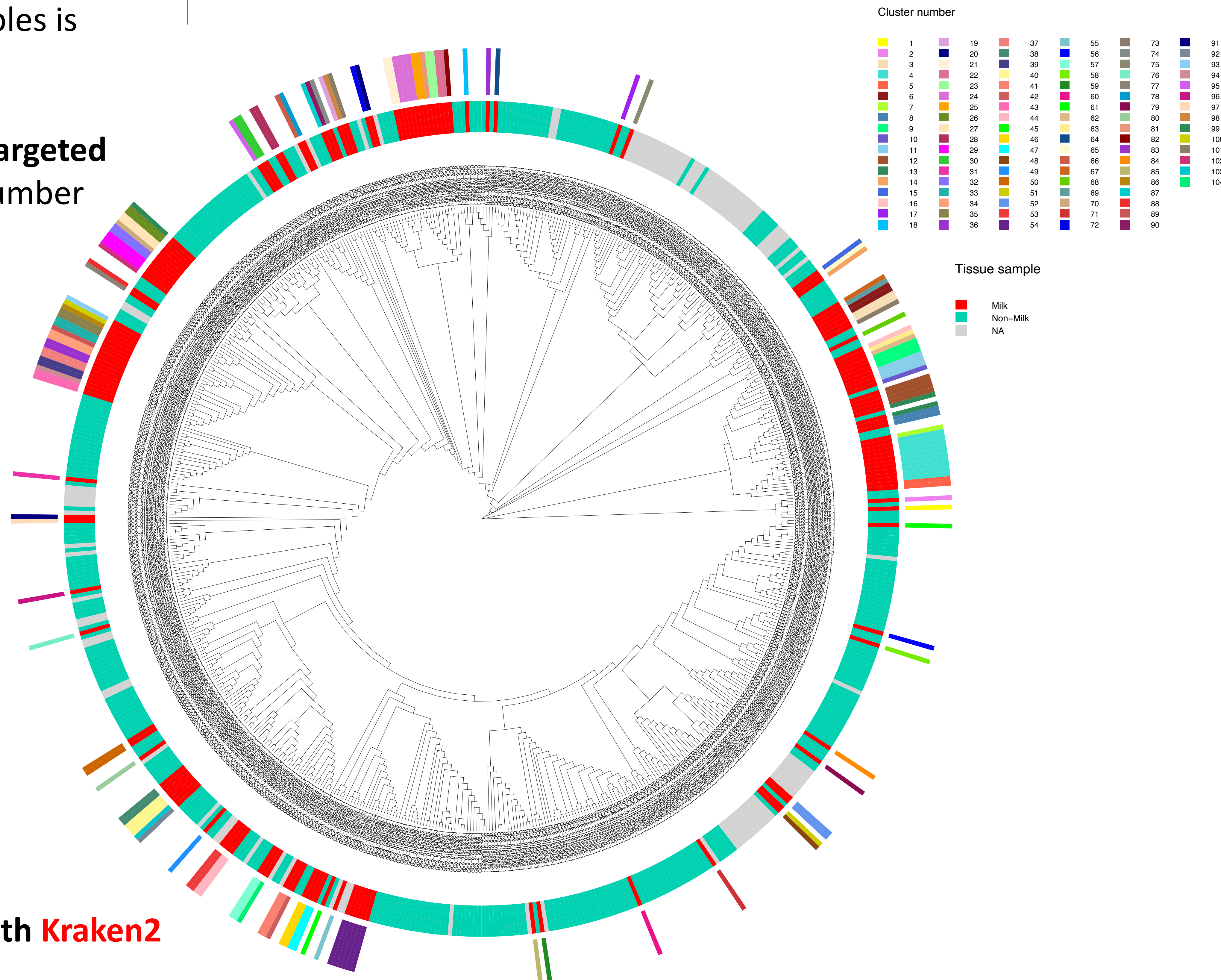
Background

Significant **variation** in *M. bovis* **transmissibility** and **disease presentations** that are **unexplained** by *species* differences or external farm management factors.

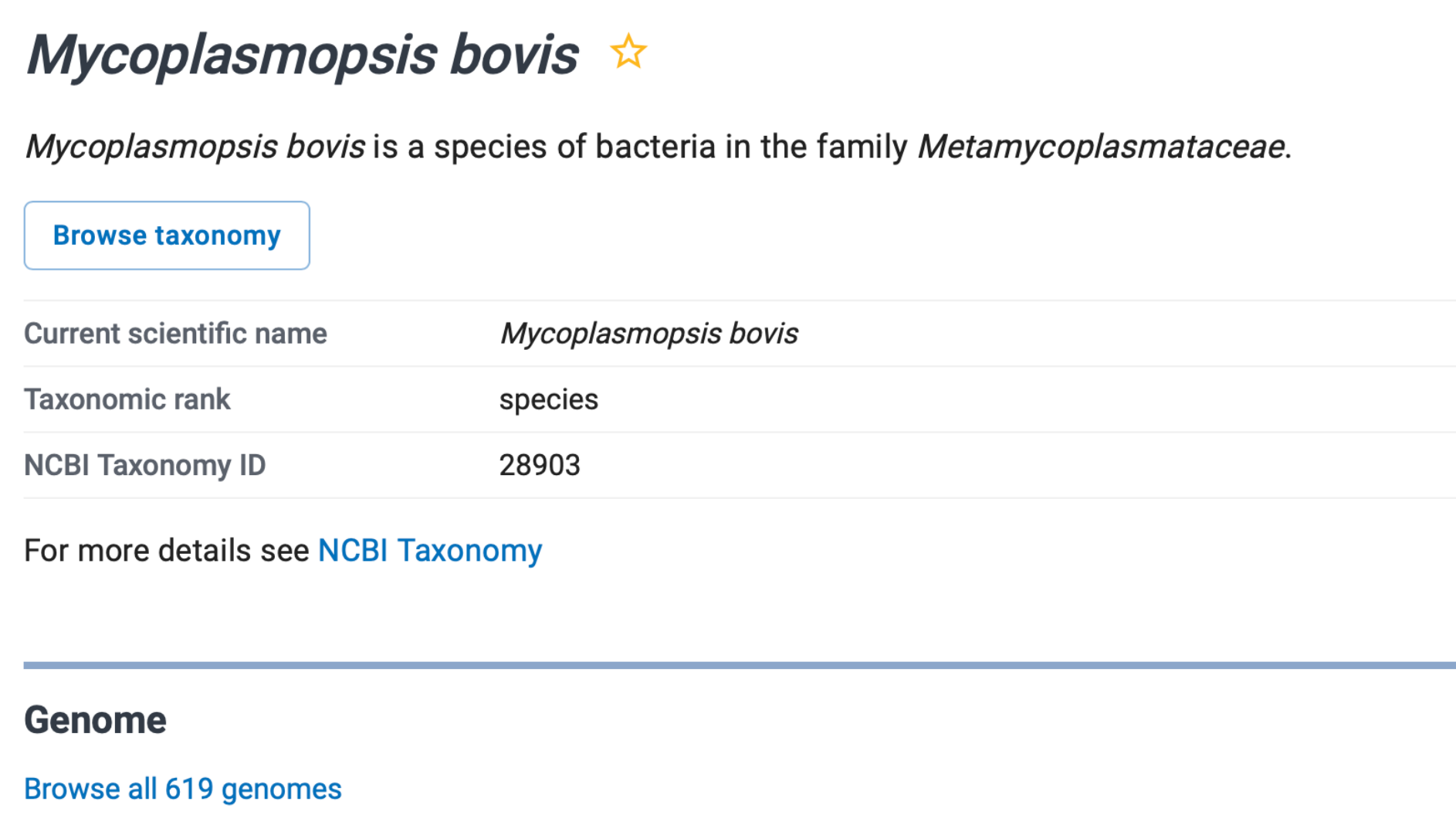
Efficient and accurate method to **distinguish** and **identify** *M. bovis* strains in metagenomics samples is **lacking**

GOAL: Evaluate the *in-silico* performance of a **targeted enriched** metagenomics method to infer the number and identity of **milk-derived *M. bovis* strains**.

2 Use **VARIANT++** pipeline in which **TreeCluster** is utilized to create a **phylogenetic tree** based on chosen thresholds for accepted **genomic similarities** and create **clusters** of genomes based on **phylogenetic cluster sequence variants (PSVs)**. Plot clusters for **milk-derived *M. bovis* genomes only**.



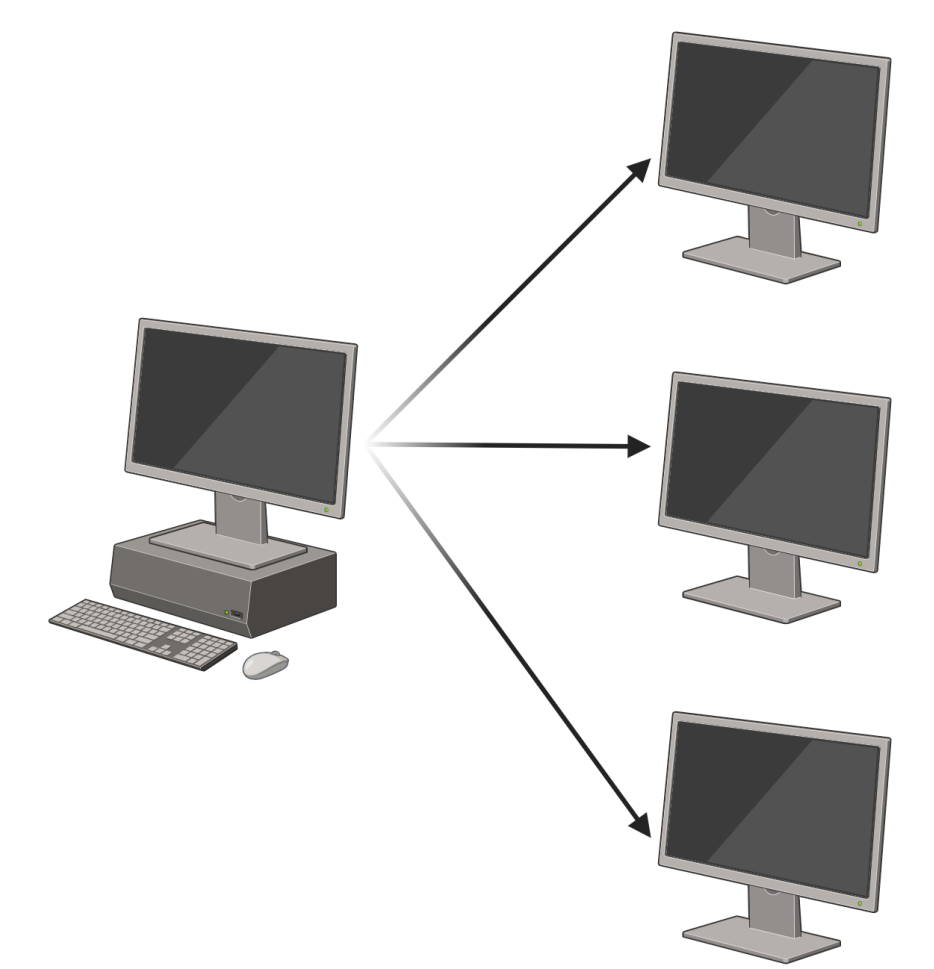
1 Download *M. bovis* genomes from NCBI



3 Build custom *M. bovis*-specific database with Kraken2

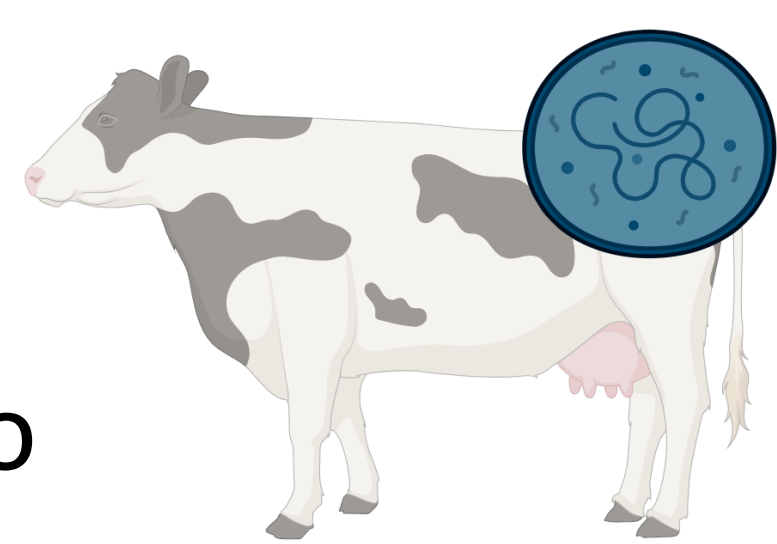
4 Simulate *M. bovis* samples using non-parametric bootstrapping based on various parameters using parallel programming in R:

1. Number of PSV's: 1, 3, 6 and 9 to model various scenarios of *M. bovis* co-infections
2. Enrichment proportion of *M. bovis*-specific reads : 30, 50, 70 and 90%
3. Number of iterations per combination: 1,000
4. Sequencing depth was randomly drawn from a **Poisson distribution** with a mean of **20 million reads**



5 Run *M. bovis* simulated samples through 2 rounds of Kraken2 (default database and custom database of step 3)

Infer the **number** and **identity** of PSVs in each **simulated sample** and **compare** with the **true values** to assess **accuracy** of method



PRELIMINARY RESULTS AND NEXT STEPS:

104 PSVs, including singletons, were detected in milk-derived *M. bovis* genomes based on the average length of the clade (threshold = 0.001). Steps 1, 2 and 3 are finalized, step 4 and 5 are in progress.

FUTURE: Apply this method to field samples taken from animals with various disease presentations and outbreak farms to determine the PSVs associated with distinct clinical outcomes and transmission characteristics.



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