

# Gall-ID: web-based tools for the rapid identification and characterization of gall-causing phytopathogenic bacteria

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## Abstract

Understanding the genetic diversity of plant pathogens and the effect of agricultural practices on pathogen evolution are important for disease management. Advances in DNA sequencing technology have contributed to greater reliance on use of 16S rDNA, multilocus sequence analysis (MLSA), and whole genome sequences to genotype bacteria. Correct analysis and interpretation of sequencing data can be difficult. Therefore we have developed a set of web-based tools, termed Gall-ID, to facilitate the identification and characterization of phytopathogenic bacteria, with a focus on those that cause gall diseases. Users can compare 16S or MLSA gene sequences from an isolate against manually-curated databases, and generate a phylogenetic tree containing their isolate. Gall-ID also includes a tool for uploading and using whole genome sequencing reads to identify homologs of known virulence genes. Finally, Gall-ID provides downloadable software pipelines for core genome analysis (WGS Pipeline), calculation of average nucleotide identity (Auto ANI), and the generation of MLSA gene set databases (Auto MLSA).

## Introduction to Gall-ID

- Web-based tools to easily identify a bacterial isolate using 16S or MLSA gene sequences
- Enables rapid identification without bioinformatics experience
- Downloadable software tools available for core genome and whole genome comparisons
- Available at:

<http://gall-id.cgrb.oregonstate.edu>

16S rDNA or MLSA  
gene sequences

I have...

Whole genome  
Sequencing reads

(online)

(downloadable tools)

Gall Isolate Typing/Phytopath-type Tools

Vir-Search

WGS Pipeline/Auto ANI

**Instructions**  
Select an MLSA dataset, input corresponding FASTA format DNA sequences for one isolate, and select submit. Copy and paste your DNA sequences in FASTA format into the window below. Sequences should be named by gene name (ie ">gyrB"). Not all genes are required to be input, though accuracy will be improved with a complete set of MLSA gene sequences. Use the options in the Analysis section to generate a Distance Tree or Minimum Spanning Network after submitting your data. These trees can be exported as pdf files or in Newick format.

Select Dataset: Choose an MLSA or 16S gene set to compare your input sequences to:  
Agrobacterium 16S [Demo]

FASTA Input

```
>16S
AACGAACGCTGGCGGAGGCTTAACACATGCAAGTCAAGCGCCCGCAAGGGGAGTGCCAGACGCGGTGATACGCGTGGAAATCTACCCA
TCTCTGGGAAATAGCTCTGGGAACTGGAATTAATACCGCATACCCCTACGGGGAAGATTATCGGGGATGGATGAGCCGGCTGTGGATT
AGCTAGTGTGGGTAAGGCCCTACCAGCGGACGATCATAGTGTCTGAGAGAGATGATCAGCCACATGGGACTGAGACACGGCCCA
ACTCCACGGAGGCGAGCTGGGGAATTTGGACAATGGGCGAAGCGCTGATCCAGCCATGCGCGTGGATGATGAAAGGCTTAGGGTTGT
AAAGCTCTTGACGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATG
AGCCTGTGCGGAAATAGCTGGGCTAAAGCCCGCCTAGGCGATGATTTAAGTCAAGCGCGGGAATCCCGAGCTCACTGGGAGCTCGCTT
ATACTGGATCTTGGATGATGAGAGAGTAAAGTGGATTCCGAGTGTAGAGGTGAATTGGATATTCGGGAAGCACCCAGTGGCAAGCG
GCTTACTGTGCTCATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAACAAGATGATACCCGTGGTGTGACGCCGTAACAAGTGAATGT
TAGCCGTGGCGAGTACTGTCTGGTGGCGGACGATTAACCAATTCCGCTGGGGAGTACGGTCCGCAAGATTAACAAGTCAAGAAT
TGACGGGGGCCCGCACAGCGGTTGGAGCAGTGTGTTAATTGCGAAGCAACGCCAGCACTACAGCTCTTGACATCGGGGATGGCGATT
GGAGCAGTGTCTTCAAGTGGCTGGCGCAGAACAGGTGCGTGTGTGCTGCGTGTGTGTGCTGCGTGTGCTGCGTGTGCTGCGTGTGCTGCG
CGAGCGAACCTCCGCCCTTAGTTGGCAGCATTAGTTGGGCACCTTAAGGGGACCTGCCGGTATAGCGAGGAGGAAAGTGGGAGTACGCT
CAAGTCTCATGGCCCTTAGCGGCTGGGCTACACAGCAGTGTGATGATGTGATGATGTGATGATGATGATGATGATGATGATGATGATGATGATG
AAAGCCATCTCAGTTCGATGCTGCACTGCACTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATG
```

Random Seed:  Choose a Distance: Kimura 80

Select an organism, a 16S or MLSA gene dataset, and input your sequences

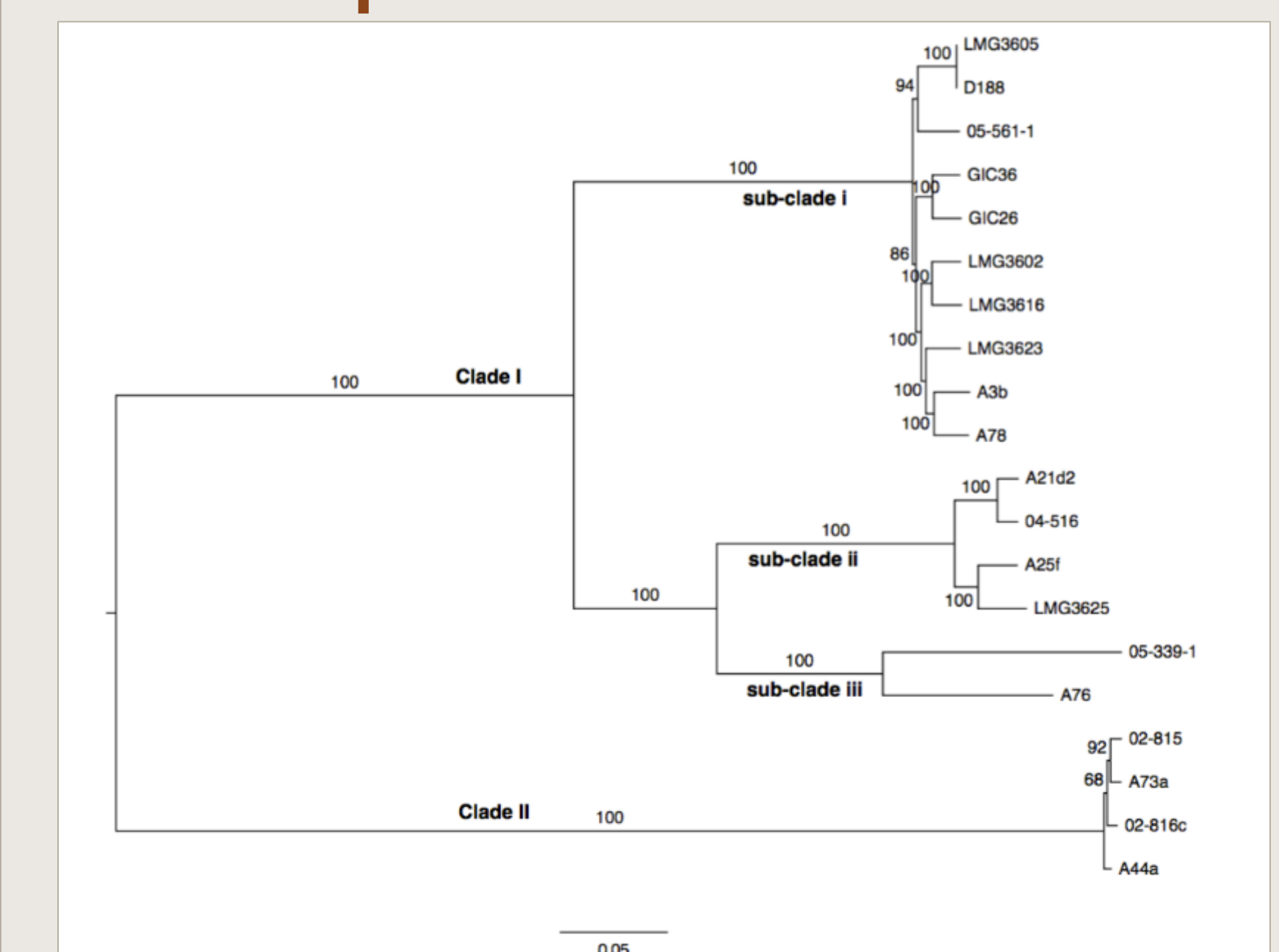
Identify the presence of known virulence genes in raw whole genome sequencing data: Gene coverage and read-mapping depth as well as the most closely related organism allele for each gene are reported.

Simplified whole genome analysis: Use whole genome sequencing reads and reference genome sequences to generate a core genome phylogeny, or calculate pairwise average nucleotide identity (ANI) between genome assemblies, with minimal user input.

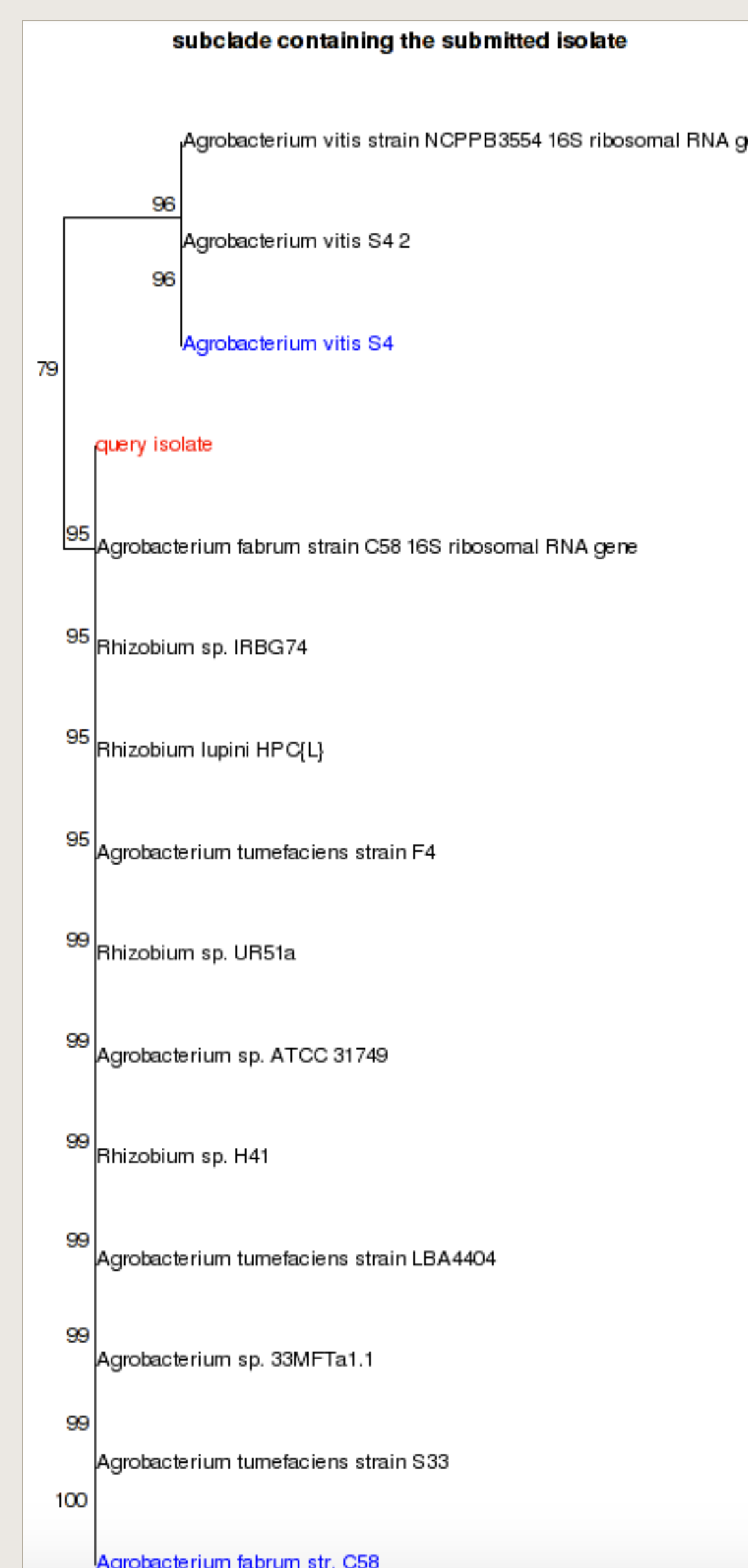
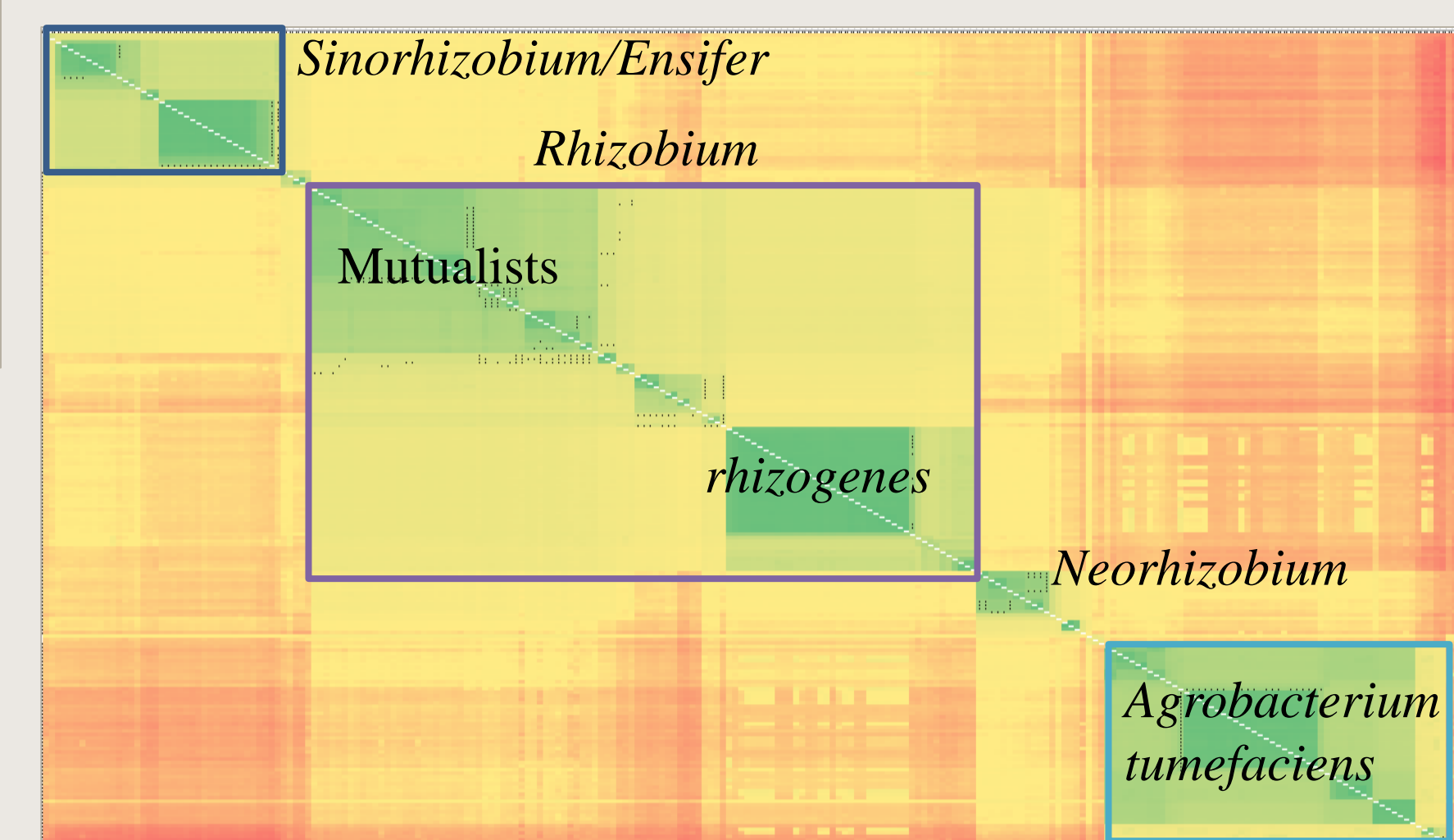
Example output of the Vir-Search tool

Found	Gene	Coverage (%)	Depth	Closest Allele
+	tms2	100.0	31.699	Rhizobium_rubi_NBRC_13261
+	tms1	100.0	27.473	Rhizobium_rubi_NBRC_13261
+	ipt	100.0	36.349	Rhizobium_rubi_NBRC_13261
x	galls	-	-	-

Phylogenetic tree of *R. fascians* genomes generated using the WGS Pipeline



Average nucleotide identity (ANI) heatmap of *A. tumefaciens* genomes generated using Auto ANI



Gall-ID produces and displays a phylogenetic tree containing your isolate for easy identification

16S database

MLSA databases:

- *Agrobacterium*
- *Clavibacter*
- *Dickeya*
- *Pantoea agglomerans*
- *Pectobacterium*
- *Pseudomonas savastanoi*
- *Ralstonia*
- *Rhodococcus fascians*
- *Xanthomonas*
- *Xylella*

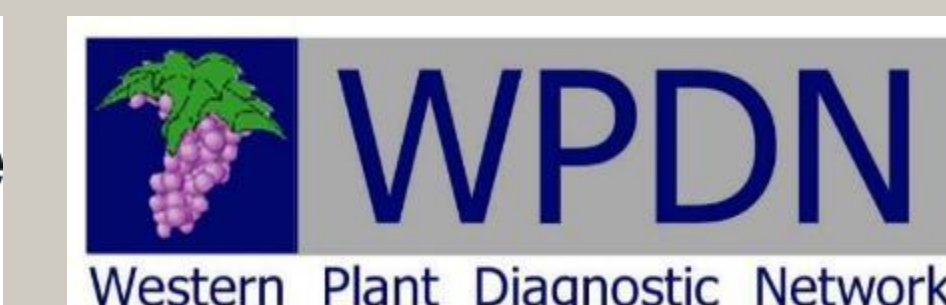
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