

Establishing a metagenomics-based pathogen identification center for plant and animal disease surveillance and diagnostics



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OVERVIEW

Virginia tech's Plant Disease Clinic and Virginia Tech Animal Laboratory Services is establishing VT-PLANS, Virigina Tech's **PL**ant and **AN**imal **S**ciences, a combined disease diagnostic and surveillance center using a metagenome-based approach to pathogen identification (Figure 1).

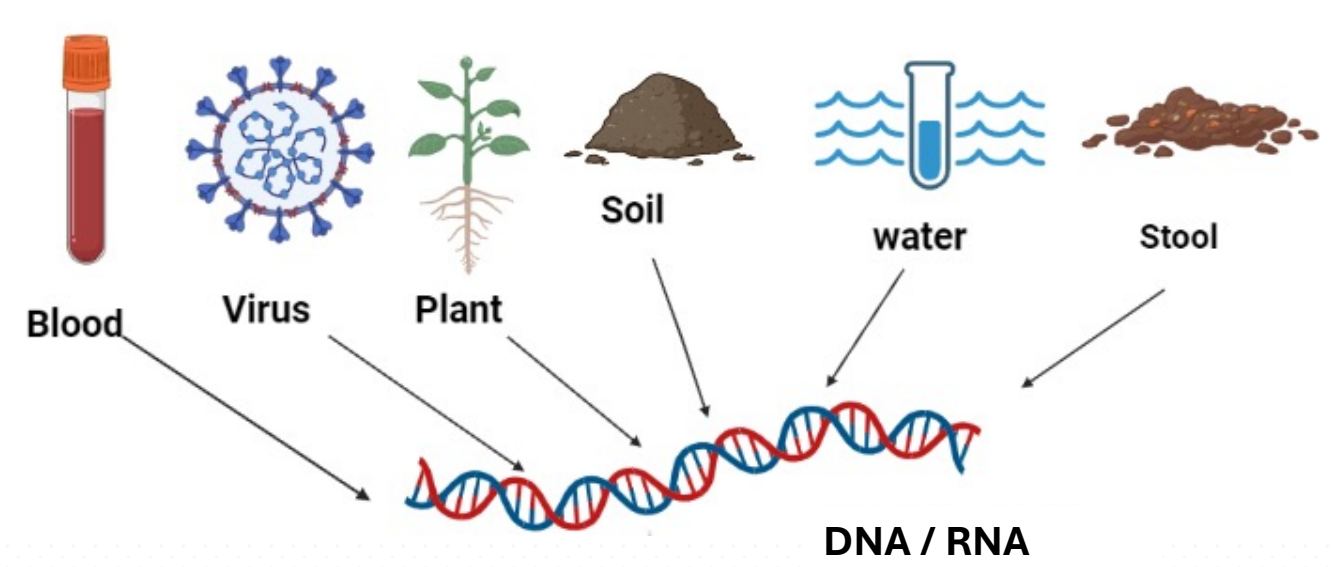


Figure 1. Genetic information can be extracted and analyzed from all types of sample sources, including both plant and animal hosts.

Advantages to VT-PLANS metagenomic approach

- Rapid method
- Organism-agnostic. Identifies all microbes in a sample
- Precise identification reaching >99.9% ANI
- Metadata tracking for long term biosecurity surveillance

Challenges to VT-PLANS

- Other microorganism can mask pathogen presence
- Imprecise classification from database contamination/artifacts
- Analysis may not be informative to surveillance

1. SUBMITTING TO VT-PLANS

Disease samples can be sent to VT-PLANS through one of our disease clinics

- Tissue
- Nucleic acid
- Sequence data

Sample metadata can be sent directly to the VT-PLANS web service or indirectly through the disease clinic

VT Plant Disease Clinic

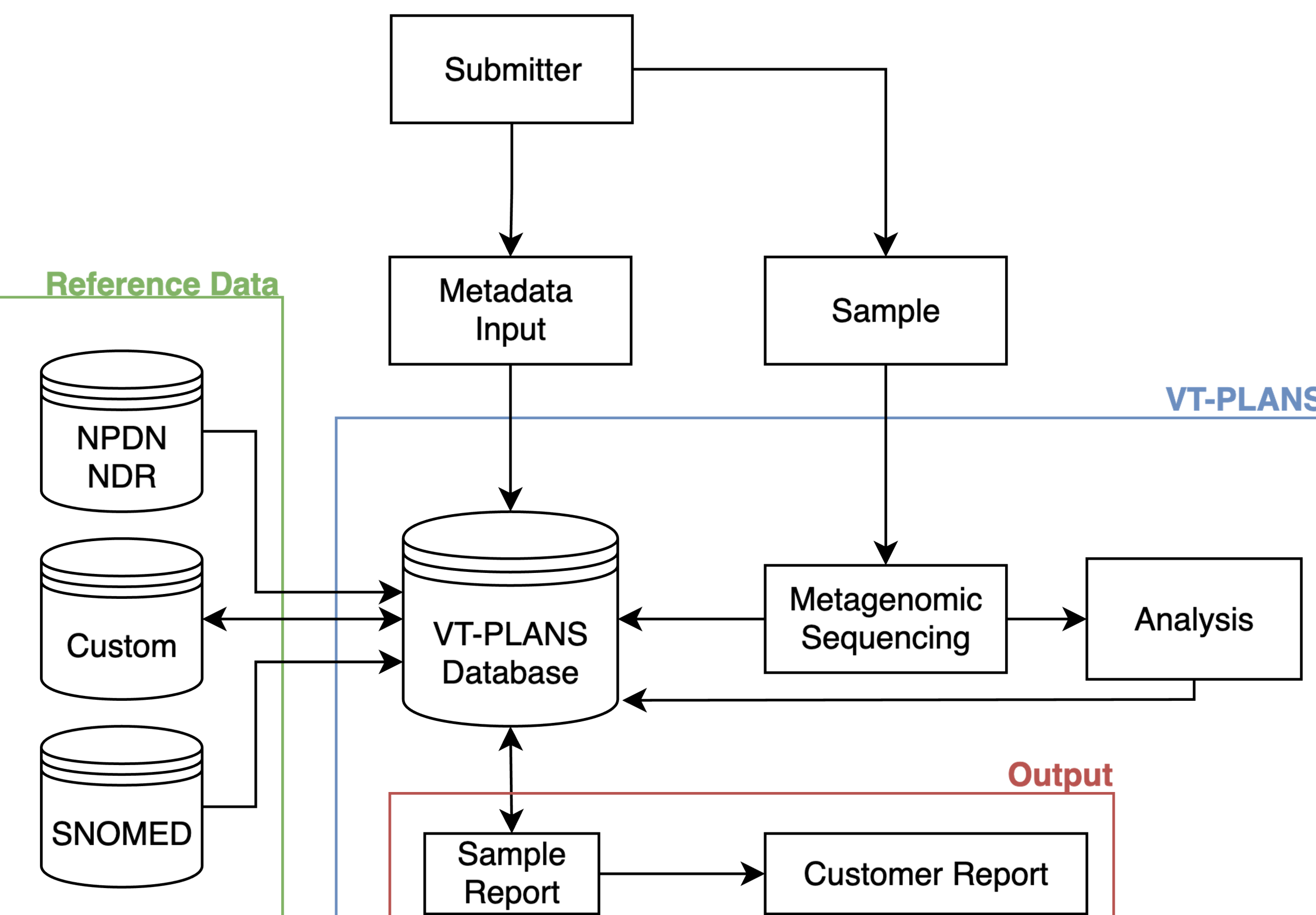


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2. METAGENOMIC WORKFLOW

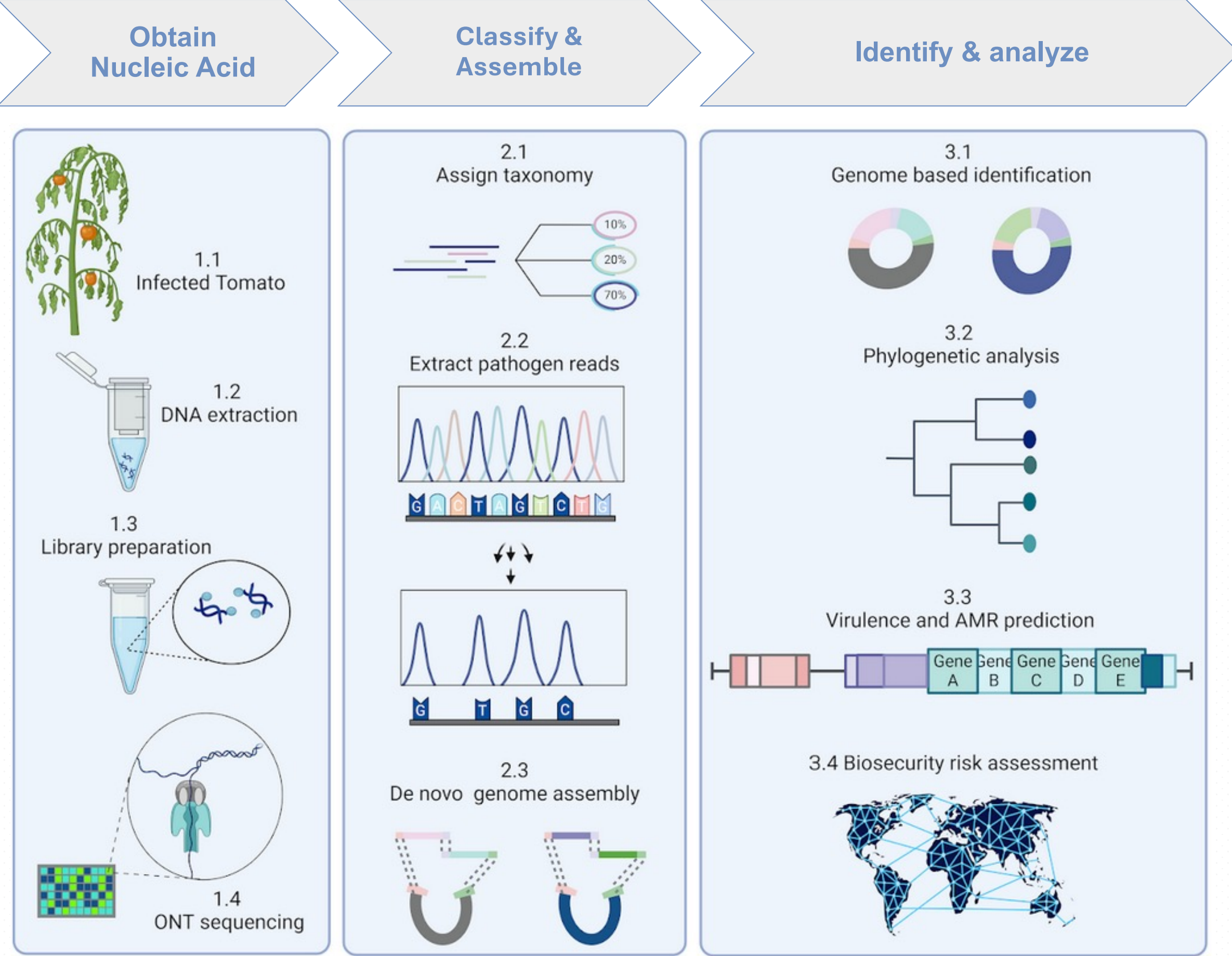


Figure 5. VT-PLANS' metagenomic workflow.

- VT-PLANS extracts nucleic acid (DNA or RNA) using tissue-specific protocols
- Oxford Nanopore Technologies' (ONT) long read sequencing allows for more accurate taxonomic read classification
 - by Kraken2¹, LINTax², or sourmash³
- Pathogen genome assembled using Flye⁴ is more precisely classified using genomeR⁵ and includes a phylogenetic analysis with related microbes
- Analysis pipeline includes virulence and AMR prediction
- All analyses used for biosecurity assessment by diagnostician

A Key strength to VT-PLANS is the use of genomeR⁵

- highly precise classification using LINs system
- Can compare with all available microbes beyond 99.9% ANI
- See P-186 poster for details



Scan here for GenomeR

INTEGRATED NATIONAL DATABASES

VT-PLANS integrates both regional and national databases for plant and animal diseases

- Track hosts and their target pathogens with up-to-date national databases (Figure 2, 3, and 4)
- Coordinate with the national diagnostic networks for biosecurity assessment (Figure 3)
- Inform national agencies on any detected emerging pathogens
- Third custom database for outliers



Figure 2. VT-PLANS collaborates with both the regional (NCPDN) and national plant diagnostic network (NPDN)

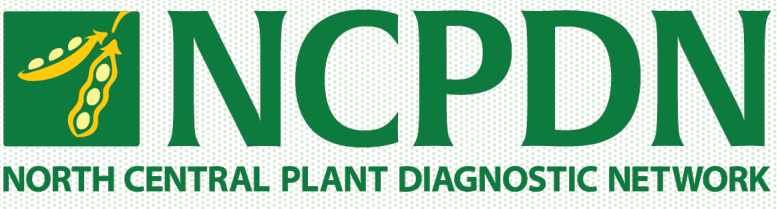
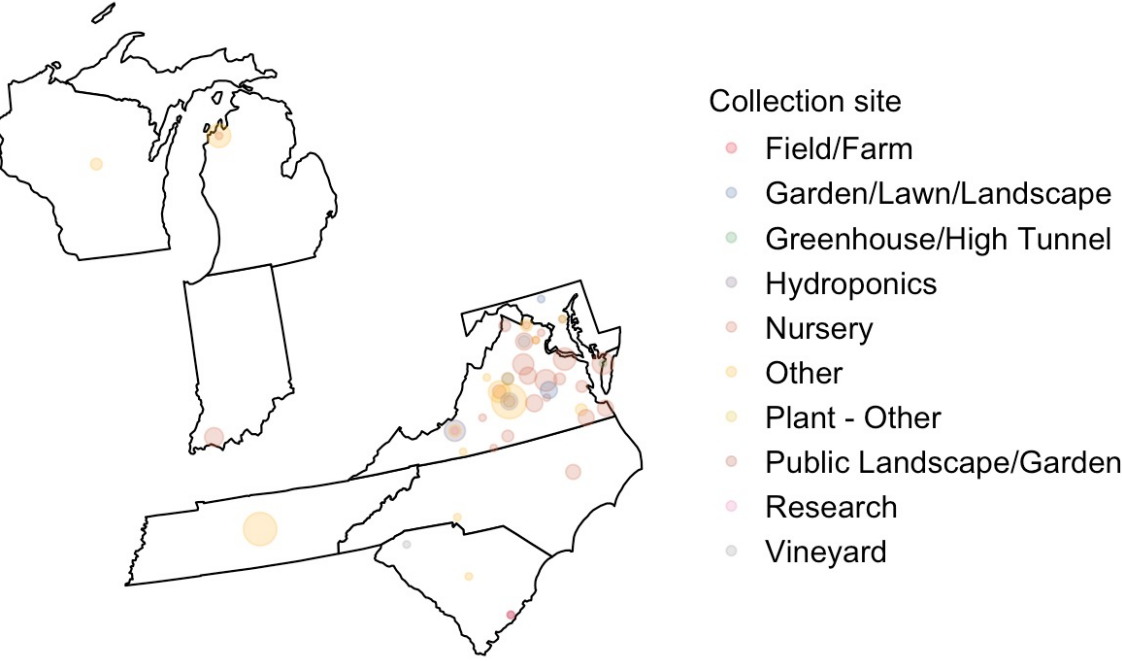


Figure 3. VT-PLANS uses the standardized database SNOMED CT for animal disease monitoring

Figure 4. Current distribution of submitted samples using generalized geolocation metadata to track disease prevalence and incidence.



References

- [1] – Lu, J. et al. (2022). Nat. Protoc. 17(12): 2815-2839.
- [2] – Sharma, P. (2024). <https://github.com/parul-sharma/LINTax>
- [3] – Irber, L. et al. (2024). J. Open Source Softw. 9(98):6830.
- [4] – Kolmogorov, M. (2019). Nat. Biotechnol. 37(5):540-546.
- [5] – Pritchard, L. et al. (2022). Access Microbiol. 4(5):po0368.
- [6] – Abdelrazek, S. et al. (2025). Phytopathol. 115(4): 354-366.

3. ANALYSIS RESULTS AND SAMPLE REPORTS

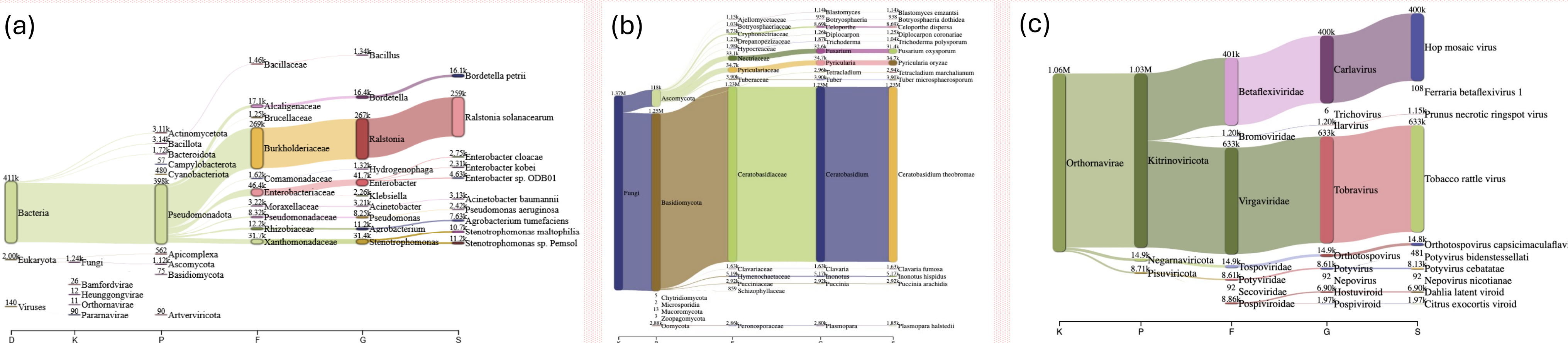


Figure 6. A variety of pathogens successfully detected by VT-PLANS using Kraken2. (a) *Ralstonia solanacearum* found in bacterial wilt tomato samples⁶. (b) Fungus *Ceratobasidium* sp. was detected in vascular streak dieback disease samples (Poster P-188). (c) Tobacco rattle virus identified in diseased tobacco plants (Poster P-187).

VT-PLANS can precisely identify a wide variety of pathogens in sequenced reads using custom databases (Figure 6).

- Generate Metagenome-assembled genomes (MAGs) from reads (Figure 7)
- Assess phylogenetic relationship with closely related genomes (Figure 8)

Users of VT-PLANS receive a sample report or a summary report.

- Sample reports provide sequencing, analysis results, and interpretations
- Summary report provide final interpretations and diagnostician recommendations

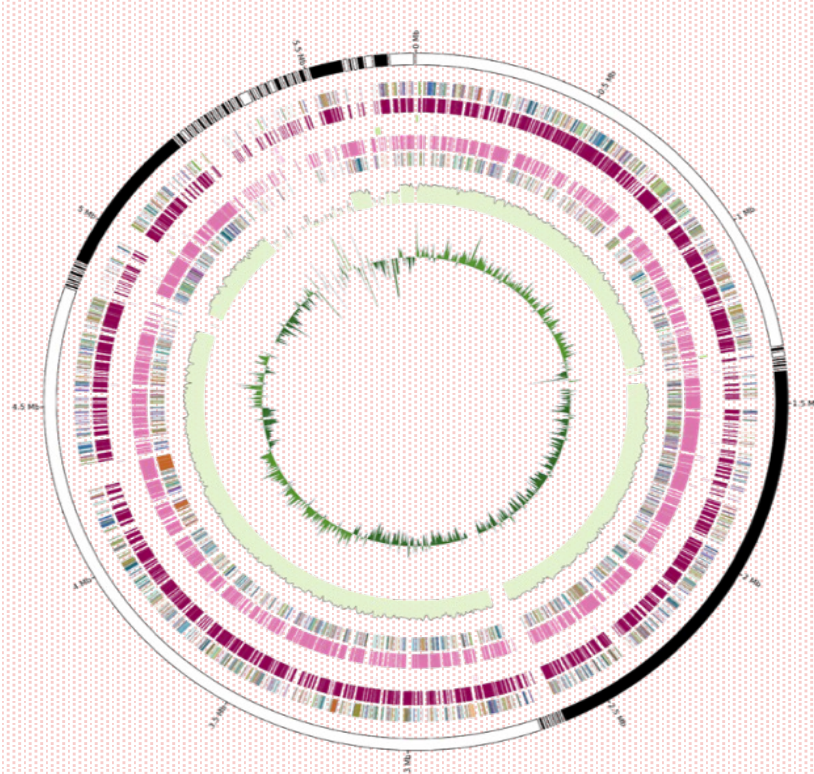


Figure 7. *R. solanacearum* MAG VA263 generated from reads⁶

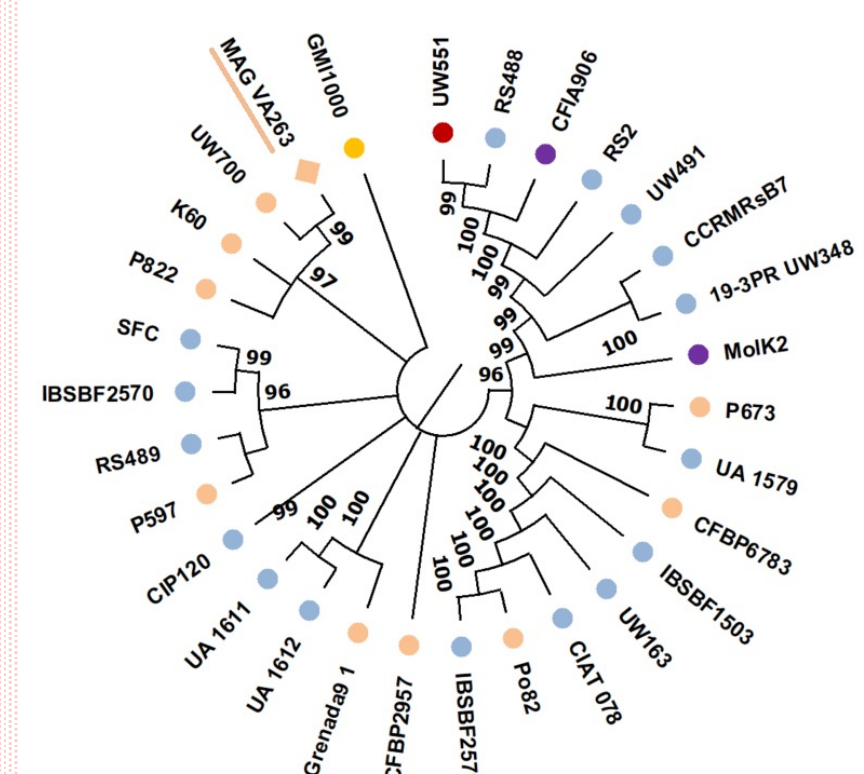


Figure 8. Phylogenetic relationship of MAG VA263 with other *R. solanacearum* strains⁶